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THE NATURE OF THE SERIES OF ENVIRONMENTAL VARIANCES AND THE ESTIMATION OF THE GENETIC VARIANCES AND THE GEOMETRIC MEANS IN CROSSES INVOLVING SPECIES OF LYCOPERSICON

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INTRODUCTION

GENETIC studies on the inheritance of quantitative characters would be materially facilitated if the nature of the series of environmental variances were known, if the genetic variances could be estimated, and if some of the problems involved in estimating the geometric means could be solved.

A knowledge of the nature of the series of environmental variances is essential to a separation of that part of the variability due to environment from that part of the variability due to the segregation of genes (genetic variability). In addition, a method whereby the genetic variance could be estimated would be particularly valuable in determining the relative importance of environment and inheritance on the development of a character, in studying the possible number of gene pairs differentiating the character under consideration, in evaluating the relative importance of so-called major and minor gene effects, and in determining the nature of the interaction of the genes involved. Accordingly, the purposes of this study are to determine the nature of the series of environmental variances, to develop a method whereby the genetic variances can be estimated, and to solve some of the problems connected with an estimation of the geometric means.

Recently, interest has been renewed in the nature of the interaction of the genes affecting quantitative characters. That the genes may have multiplicative effects was suggested by EAST in 1913. More recently WRIGHT (1922), DALE (1929), POWERS (1934, 1936, 1939a, 1939b, 1941), POWERS and LYON (1941), LINDSTROM (1935), HOUGHTALING (1935), SMITH (1937), MACARTHUR and BUTLER (1938), CURRENCE (1938) and CHARLES and SMITH (1939) have published papers on the nature of the interactions of the genes affecting quantitative characters. From these studies, it would seem that the nature of the interactions of the genes affecting quantitative characters, to a considerable extent, is dependent upon the character and material being studied. It has been definitely shown by POWERS (1941) that in some cases the gene effects are additive while in others they are geometrically cumulative.

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EXPERIMENTAL DESIGN AND REDUCTION OF THE DATA

Weights of individual tomato fruits reported on in this paper were taken from the cross of Danmark (*Lycopersicon esculentum* Mill.×Red Currant [*L. pimpinellifolium* (Jusl.) Mill.]. The weights were taken in grams and transformed to logarithms. Hence, unless otherwise stated, all data are expressed as logarithms. Both parents, the F_1 generation, the F_2 generation and the backcross generations were grown in a complete randomized block experiment including ten blocks with two replications per block. The parents and generations were randomized within replications. To provide that the variants also occur at random within blocks, 20 different sets of numbers taken from Tippett's Tables were used in randomizing the replications. The method of reducing the data was the same as that employed by POWERS (1939a) with the exception that as regards the segregating generations the calculations were within generations rather than within genotypes of a given generation. The reason for making generations rather than the genotype the basis for the calculations was to insure that the variances within blocks would include the genetic variability. It should be understood that the variability within blocks does not form a basis of calculating measures of reliability for comparing generation and parental means since the "within block" variances, both environmental and genetic, are based on individual plant data and include the variability due to replications. That is, the calculations are for "within blocks" ignoring replications. The standard deviations used in determining the significance of differences between the means of generations and parents were calculated from plot means, and the variation due to replications is removed. The estimate of error is the interaction between blocks and replications. The method employed in analyzing the data is illustrated by the two analysis summaries given in table 1. The "within block" variances, both environmental and genetic, are based on individual plant data. The symbol B_1 which will appear both in the tables and in the text signifies that the progeny studied resulted from back-crossing the F_1 to the designated parent. In all calculations the data were carried to six places to the right of the decimal point. However, the constants given in the tables may be contracted to fewer than six places.

METHODS AND EXPERIMENTAL DATA

The percentage of the genetic variance included in the variance for "between means of blocks"

For purposes of genetic analysis, variation may be classified into two categories—namely, that due to environment (environmental variation) and that due to segregation of genes (genetic variation). Since one of the purposes of this study was to devise a method of determining that portion

of the variances attributable to each cause, it is desirable to examine the data and analysis summaries given in table 1 with that idea in mind. Also, from an examination of this table it may be seen that the environmental variances and genetic variances were calculated from individual plant data and are the "within block" variances. An appreciation of these facts is basic to an interpretation of the data. Moreover, these facts should be kept in mind so as not to confuse these variances with those used in calculating the standard errors employed as a measure of reliability of the means of generations and parents. From the first summary analysis of table 1 it may be seen that the total variation has been separated into two parts—namely, that attributable to differences between means of blocks and that within blocks. As in all such designs (FISHER 1937), the purpose of dividing the experimental area into blocks was to control that portion of the variability ascribable to block means. However, before eliminating the variability attributable to differences between means of blocks one must ascertain whether or not that amount of the variation ascribable to the segregation of genes and confounded with environmental differences between block means is negligible. If an amount of the genetic variability sufficient to influence an interpretation of the data is included with the differences between means of blocks, it is apparent that the variability due to differences between means of blocks must be included in the study designed to determine the variance attributable to the segregation of genes.

TABLE 1

Analysis summary of weight of fruit for the B_1 to Danmark. In the analysis individual plant data expressed in grams were transformed to logarithms.

VARIATION DUE TO·	D.F.	SUMS OF SQUARES	MEAN SQUARE
		Within blocks	
Total	933	38.359746	0.041114
Between means of blocks	9	3.435507	.381723
Within blocks	924	34.924239	.037797
		Blocks and replications	
Total	19	0.097327	0.005122
Between means of blocks	9	.076525	.008503
Between means of replications	1	.005107	.005107
Blocks×replications	9	.015695	.001744

Since such is the case, it is necessary to ascertain the proportion of the genetic variability that is included in the differences between means of blocks. It is apparent that if only one individual of any segregating generation were grown per block, all the genetic variability would have been included in the variation ascribable to blocks. On the other hand, if the infinite population were included in each block, all the genetic variability

would be within blocks and none included with the variability due to differences between means of blocks. These considerations show that the proportion of the genetic variability that is included in the differences between block means is dependent upon the value of "n." The percentages of the genetic variance included in the variance for "between means of blocks" for values of "n" up to 100 are given in figure 1. The graph presented in figure 1 was constructed by use of the formula $Vg\bar{x} = V/n$, in which $Vg\bar{x}$ is that proportion of the genetic variance included in the vari-

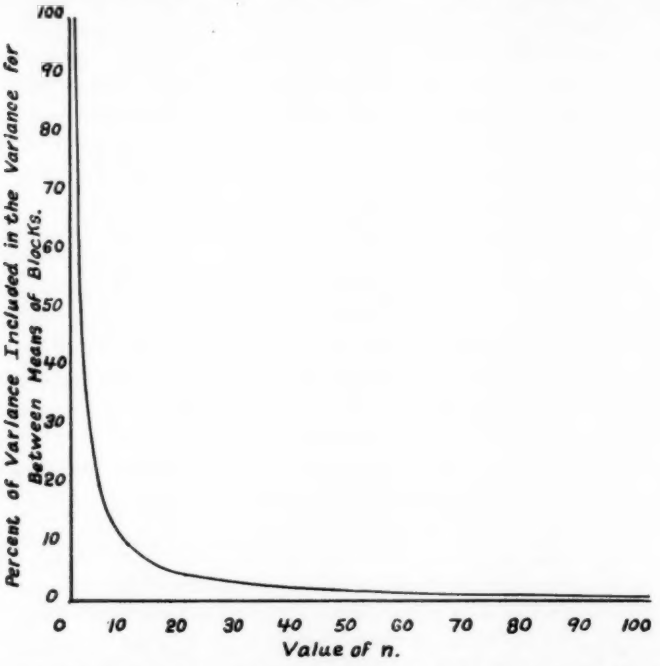

FIGURE 1.—Decrease in the percentage of the genetic variance included in the variance for between means of blocks, as the value of n increases.

ance for any particular block mean, V is the genetic variance of a single determination calculated from individual plant data and is expressed as 100 percent, and n is the number of plants of any particular generation grown per block. From this figure it may be seen that when the number of individuals per block is 90, the percentage of the genetic variance included in the variance for "between means of blocks" is only slightly over 1 percent. In the present study (see table 1) the average value of "n" per block for the B_1 to Danmark was 93.4. The average number of individuals per

block for the B_1 to Red Currant and the F_2 generation was 93.2. Hence, as far as this study is concerned the proportion of the genetic variance included in the variance for "between means of blocks" is so small that it would not have any effect upon an interpretation of the results. Therefore, the remainder of the study will be confined to the "within block" variance. However, it should be pointed out before leaving this subject that in those studies where it is desirable to do so, once that portion of the genetic variance for "within blocks" has been ascertained, the total genetic variance can be estimated by making allowances for that portion which has been included in the variance for "between means of blocks." The theoretical amount included in the variance for "between means of blocks" can be obtained for the ordinate values of figure 1, if the number of individuals does not exceed 100.

THE NATURE OF THE SERIES OF ENVIRONMENTAL VARIANCES

To obtain information concerning the nature of the series of environmental variances, the means and the total "within block" variances of the generations and parents were listed in columns 2 and 3 of table 2. In this table the data on weight of fruit are expressed in grams. It may be seen from the data in columns 2 and 3 of table 2 that the magnitudes of the variances and the magnitudes of the means are associated. Hence, it would seem that the laws governing the formation of the series of environmental variances may be such that these variances form a geometric progression. Since the genetic variability for the Red Currant parent, the F_1 generation and the Danmark parent is negligible, their variances may be used to determine whether or not the environmental variances form a geometric progression. If they do form a geometric progression, the logarithms of weight of fruit should be normally distributed (ZELENY 1920; WRIGHT, 1922, 1926; DALE 1929; SMITH 1937). Then, if the variances are proportional to the means, a straight line relationship should exist between the two.

The total number of individuals per population, the means and the variances are given in table 3. Starting with the Red Currant parent, as in the table, the means of the variants are represented by $x_1 \cdots x_6$ and the total variances are represented by $y_1 \cdots y_6$, respectively. As previously pointed out from an examination of the means and environmental variances of the parents and F_1 generation it would seem that the variances are proportional to the means and, hence, that a straight line relationship might exist between these two variables. If such is the case, the predicted environmental variance of the F_1 generation should not be significantly different than the obtained value. Then, $y = mx + b$ (any college algebra text); in which y is the predicted variance, m is the slope of the line, x is the corresponding mean of the predicted variance, and b is the variance

for which x equals zero. An examination of table 3 shows that three equally appropriate values of m can be calculated—namely, $m = y_6 - y_1/x_6 - x_1$, $m = y_3 - y_1/x_3 - x_1$, and $m = y_6 - y_3/x_6 - x_3$, on the basis that the co-ordinates (x_1, y_1), (x_3, y_3), and (x_6, y_6) are points on a straight line. In this study the m employed is the average of the three calculations, and $b = y_1 - mx_1$.

TABLE 2

The means and "within block" variances for weight of fruit (expressed in grams) for the generations and parents.

GENERATION OR PARENT	MEANS	TOTAL VARIANCES
Red Currant	0.915	0.053
B_1 to Red Currant	2.095	.743
F_1 generation	5.480	2.258
F_2 generation	5.380	10.032
B_1 to Danmark	16.105	50.940
Danmark	51.170	236.942

For the sake of clarity, it might be well to follow through in detail the calculation of the predicted F_1 variance. In obtaining m we have,

$$m_1 = \frac{y_6 - y_1}{x_6 - x_1} = \frac{0.020967 - 0.012860}{1.671515 + 0.053440} = 0.004700,$$

$$m_2 = \frac{y_3 - y_1}{x_3 - x_1} = \frac{0.017856 - 0.012860}{0.707441 + 0.053440} = 0.006566,$$

$$m_3 = \frac{y_6 - y_3}{x_6 - x_3} = \frac{0.020967 - 0.017856}{1.671515 - 0.707441} = 0.003227,$$

and the average value of $m = 0.004831$. Then, by substituting the proper values in the formula, $b = 0.012860 - (0.004831 \times -0.053440) = 0.013118$. Now, from the general formula $y_3 = mx_3 + b$, we have, by substitution, $(0.004831 \times 0.707441) + 0.013118 = 0.016536$, which is the predicted variance for the F_1 generation.

The obtained and predicted environmental variances for the F_1 generation are listed in columns 6 and 7 of table 3. Tests by a method furnished by W. G. COCHRAN (personal correspondence) show that the difference between the obtained and predicted variances of the F_1 generation can be attributed to chance variation. Hence, the data are in agreement with the supposition that the series of environmental variances, calculated from individual plant data on weight of fruit expressed in grams, form a geometric progression and are proportional to the means.

Such being the case, these data fall under the category of those discussed as follows by WRIGHT (1926):

"We have assumed above that a given elementary factor always makes

the same contribution to the total effect. There is often reason to believe, however, that in natural variation, the effect of one factor may depend on the presence and effect of other factors and especially on the total effect of the latter. In other words, the scale on which the variates are measured may not be in harmony with the nature of the factors. This type of complication is likely to produce wider departures from normality than those due to differences among the factors in chance of occurrence, amount of contribution, or correlation in occurrence.

"The logical method of dealing with variation due to such factors is, as has frequently been pointed out, to transform the scale to one on which each factor has the same effect throughout the range. Practically, the method is usually to find the transformation which will reduce the family of distributions which is under study, to normal distributions."

Then, from this discussion by WRIGHT and from the work of ZELENY (1920) it is evident that the logical method of analyzing these data is to transform the individual plant weights of fruit to logarithms and use them in the calculations. As previously pointed out, this procedure was followed in the present study.

TABLE 3

Symbols, means, and variances of logarithms for weights of fruit.

PARENT OR GENERATION	N	MEAN		VARIANCES			
		SYMBOL	VALUE	SYMBOL	TOTAL	ENVIRONMENTAL	GENETIC
Red Currant	420	x_1	−0.053440	y_1	0.012860		
B_1 to Red Currant	932	x_2	.286961	y_2	.028038	0.014504	0.013534
F_1 generation	475	x_3	.707441	y_3	.017856	.016536	
F_2 generation	932	x_4	.672012	y_4	.048285	.016364	.031921
B_1 to Danmark	934	x_5	1.161870	y_5	.037797	.018731	.019066
Danmark	456	x_6	1.671515	y_6	.020967		

GENETIC VARIANCE

The genetic variances are given in column 8 of table 3. These values were obtained by subtracting the environmental variances from the total variances. In the present study these variances are of primary interest because of the bearing they have upon the problem of genic dominance. A comparison of the means of the Red Currant parent, the F_1 generation, and the Danmark parent shows that phenotypic dominance exists. That is, the mean of the logarithms for weight of fruit of the F_1 generation is closer to the mean of the Red Currant parent than it is to the mean of the Danmark parent. If genic dominance is present also, the genetic variance of the B_1 to Red Currant would be expected to be less than the genetic variance of

the B_1 to Danmark. Column 8 of table 3 shows that such is the case, and tests by W. G. COCHRAN (personal correspondence) show that this difference is greater than would be expected to occur by chance. The evidence is rather convincing in support of the contention that genic dominance occurs in the cross involving Danmark and Red Currant.

ESTIMATION OF THE GEOMETRIC MEANS

Before using the formulas given for estimating the geometric means of the segregating generations, it is necessary to know whether or not the data being analyzed conform to the fundamental genetic suppositions basic to the development of the formulas. CHARLES and SMITH (1939) give formulas for predicting the means and variances of the F_1 and segregating generations together with the proof of the validity of these formulas. This proof shows that their formulas are applicable when there is no genic dominance when the effects attributable to the differences between contrasted alleles are of equal magnitude. These formulas given by CHARLES and SMITH (1939) are not applicable to the data on weight of fruit of the parents and generations of the Danmark and Red Currant cross, because there is genic dominance and, as will be shown in another publication, the differences between contrasted alleles are not all of the same magnitude.

The formulas for predicting the means of the segregating generations given by WRIGHT, see POWERS (1939a), are as follows:

$$B_1 \text{ to } P_1 \text{ (dominant parent)} = \frac{\log \overline{P}_1 + \log \overline{F}_1}{2}$$

$$F_2 \text{ generation} = \frac{\log \overline{P}_1 + 2 \log \overline{F}_1 + \log \overline{P}_2}{4}$$

$$B_1 \text{ to } P_2 \text{ (dominant parent)} = \frac{\log \overline{F}_1 + \log \overline{P}_2}{2}.$$

Before using these formulas, it is necessary to know under what genetic conditions they are applicable. In other words we need to test whether these formulas are applicable irrespective of the intra-allelic interactions (dominance relationships), the relative magnitudes of the effects attributable to the different gene pairs, the number of gene pairs differentiating the character under consideration, and finally whether the gene pairs involved are independently inherited, are linked, or some are linked and others are independently inherited. In testing whether or not WRIGHT's formulas are applicable irrespective of the above genetic assumptions, the fundamental theories developed by previous investigators (WRIGHT 1922; DALE 1929; SMITH 1937; MACARTHUR and BUTLER 1938; CHARLES and SMITH 1939)

were used. The paper by WRIGHT (1922) and the paper by CHARLES and SMITH (1939) were particularly helpful.

Before going into the detailed test of the formulas and to avoid confusion, some matters of terminology, symbols, and abbreviations should be clarified. Those genes common to both parents and which affect the character under consideration will not be included in the discussions, since the conclusions drawn would not be altered by so doing. Throughout the discussion when the term mean is used it will always refer to the means of the logarithms for weight of fruit and not the actual weights expressed in grams, unless so designated. The symbol P_1 will be used to designate the dominant parent and P_2 the recessive parent. In formulas and occasionally in the main body of the text the word logarithm will be abbreviated to log. Likewise, $\overline{F}_1$, $\overline{P}_1$, and $\overline{P}_2$ unless otherwise designated will refer to the means of the logarithms and not to the logarithms of the means expressed in grams. Throughout, the effects of the genes will be treated on the basis of any given gene pair (KK, and Kk and kk) rather than on the basis of any given gene (K and k). Later in the article, different formulas will be given for predicting the theoretical means of the segregating generations. At this time the terminology applied to each should be given. The formulas based on the theoretical genotypes of the parents and the F_1 generation and which are applied in predicting the theoretical means of the different generations will be referred to as the formulas for the theoretical predicted means. These are the formulas provided by WRIGHT. The formulas for calculating the theoretical means of the segregating generations based on the assumption that these generations are composed of certain genotypes will be termed the formulas for determining the theoretical means of the segregating generations. The means calculated by substituting the obtained means of the logarithms of the F_1 generation and parents in the formulas for deriving the theoretical predicted means of the different generations will be referred to as the predicted means of the segregating generations. Finally, means calculated from the logarithms of the segregating generations will be referred to as the obtained means of these generations.

There are two simple algebraic laws which are used repeatedly in the derivation of proofs—namely, that the addition of logarithms is equivalent to the multiplication of the original figures and that addition is associative. For example, if the effects of the gene pairs are multiplicative, the expression(AABB+AABb+AAbb+AaBB+AaBb+Aabb+aaBB+aaBb +aabb) becomes (log AA+log BB)+(log AA+log Bb)+(log AA+log bb)+(log Aa+log BB)+(log Aa+log Bb)+(log Aa+log bb)+(log aa +log BB)+(log aa+log Bb)+(log aa+log bb). Since addition is associative, by collecting like terms, this last expression may be written in the following form: log AA+2 log Aa+log aa+log BB+2 log Bb+log bb.

With this terminology and these facts in mind, tests may be made to determine whether or not WRIGHT's formulas are applicable under the gentic conditions specified previously. These formulas will be applicable, if the formulas for deriving the theoretical predicted means and the formulas for deriving the theoretical means are identities, irrespective of the genetic phenomena stipulated above. The problem has been reduced to one of testing whether or not such is the case.

In attacking this problem let K_1k_1, K_2k_2, $\cdots$, K_nk_n represent pairs of contrasted alleles. Then the genotypes of the parents and F_1 generation may be depicted as follows: $P_1 = K_1K_1\ K_2K_2 \cdots K_nK_n$; $F_1 = K_1k_1\ K_2k_2 \cdots K_nk_n$; $P_2 = k_1k_1\ k_2k_2 \cdots k_nk_n$. Substituting these values in the formulas for the theoretical predicted means gives,

$$B_1 \text{ to } P_1 = \frac{\log (K_1K_1K_2K_2 \cdots K_nK_n) + \log (K_1k_1K_2k_2 \cdots K_nk_n)}{2}$$

$$F_2 \text{ generation} = \frac{\log (K_1K_1K_2K_2 \cdots K_nK_n) + 2 \log (K_1k_1K_2k_2 \cdots K_nk_n) + \log (k_1k_1k_2k_2 \cdots k_nk_n)}{4}$$

$$B_1 \text{ to } P_2 = \frac{\log (K_1k_1K_2k_2 \cdots K_nk_n) + \log (k_1k_1k_2k_2 \cdots k_nk_n)}{2}.$$

By applying the algebraic laws that the addition of logarithms is equivalent to the multiplication of the original figures and that addition is associative and then collecting like terms, these formulas become,

$$B_1 \text{ to } P_1 = \frac{\log K_1K_1 + \log K_1k_1 + \log K_2K_2 + \log K_2k_2 + \cdots + \log K_nK_n + \log K_nk_n}{2}$$

$$F_2 \text{ generation} = \frac{\log K_1K_1 + 2 \log K_1k_1 + \log k_1k_1 + \log K_2K_2 + 2 \log K_2k_2 + \log k_2k_2 + \cdots + \log K_nK_n + 2 \log K_nk_n + \log k_nk_n}{4}$$

$$B_1 \text{ to } P_2 = \frac{\log K_1k_1 + \log k_1k_1 + \log K_2k_2 + \log k_2k_2 + \cdots + \log K_nk_n + \log k_nk_n}{2}$$

From these formulas it can be seen that the addition of any gene pair (Kk) makes the following addition to the existing formula,

$$B_1 \text{ to } P_1 \text{---addition of } \frac{\log KK + \log Kk}{2}$$

$$F_2 \text{ generation---addition of } \frac{\log KK + 2 \log Kk + \log kk}{4}$$

$$B_1 \text{ to } P_2 \text{---addition of } \frac{\log Kk + \log kk}{2}$$

Next consider the formulas for determining the theoretical means of the segregating generations. Let Kk be any given pair of alleles differentiating the character under consideration and $U_1, U_2, \cdots, U_n$ the genotypes with which KK, and Kk and kk may be combined. From genetic principles the number of individuals in the theoretical population is known to be 2^n for backcross generations and 4^n for the F_2 generation, in which "n" is the number gene by pairs by which the contrasted characters are differentiated. Then, the theoretical population may be depicted as follows:

$$B_1 \text{ to } P_1 = [KKU_1 + KKU_2 + \cdots + KKU_n] + [KkU_1 + KkU_2 + \cdots + KkU_n]$$

$$F_2 \text{ generation} = [KKU_1 + KKU_2 + \cdots + KKU_n] + 2[KkU_1 + KkU_2 + \cdots + KkU_n] + [kkU_1 + kkU_2 + \cdots + kkU_n]$$

$$B_1 \text{ to } P_2 = [KkU_1 + KkU_2 + \cdots + KkU_n] + [kkU_1 + kkU_2 + \cdots + kkU_n]$$

Since the addition of logarithms is equivalent to the multiplication of the original figures these formulas become,

$$B_1 \text{ to } P_1 = [(\log KK + \log U_1) + (\log KK + \log U_2) + \cdots + (\log KK + \log U_n)] + [(\log Kk + \log U_1) + (\log Kk + \log U_2) + \cdots + (\log Kk + \log U_n)]$$

F_2 generation

$$= [(\log KK + \log U_1) + (\log KK + \log U_2) + \cdots + (\log KK + \log U_n)] + 2[(\log Kk + \log U_1) + (\log Kk + \log U_2) + \cdots + (\log Kk + \log U_n)] + [(\log kk + \log U_1) + (\log kk + \log U_2) + \cdots + (\log kk + \log U_n)]$$

$$B_1 \text{ to } P_2 = [(\log Kk + \log U_1) + (\log Kk + \log U_2) + \cdots + (\log Kk + \log U_n)] + [(\log kk + \log U_1) + (\log kk + \log U_2) + \cdots + (\log kk + \log U_n)].$$

It should be noted that in all these formulas the terms between any given set of parentheses represent an individual plant of the theoretical population. Then, the total number of individuals in the backcross populations is $2(2^n)$ and the frequency of KK plants and Kk plants in the B_1 to P_1 is 2^n. Likewise, the frequency of Kk plants and kk plants in the B_1 to P_2 is 2^n. The total number of individuals in the F_2 generation is $4(2^n)$ and the frequency of KK plants and kk plants is 2^n, and of Kk plants is $2(2^n)$. Now since addition is associative, these formulas become

$$B_1 \text{ to } P_1 = 2^n[\log KK + \log Kk] + [\log U_1 + \log U_2 + \cdots + \log U_n]$$

and the mean is

$$2^n\frac{[\log KK+\log Kk]}{2(2^n)}\quad\frac{[\log U_1+\log U_2+\cdots+\log U_n]}{2}=\frac{\log KK+\log Kk}{2}+\frac{\log U_1+\log U_2+\cdots+\log U_n}{2}$$

$$F_2\text{generation}=2^n[\log KK+2\log Kk+\log kk]+[\log U_1+\log U_2+\cdots+\log U_n]$$

and the mean is

$$\frac{\log KK+2\log Kk+\log kk}{4}+\frac{\log U_1+\log U_2+\cdots+\log U_n}{4}$$

$$B_1\text{ to }P_2=2^n[\log Kk+\log kk]+[\log U_1+\log U_2+\cdots+\log U_n]$$

and the mean is

$$\frac{\log Kk+\log kk}{2}+\frac{\log U_1+\log U_2+\cdots+\log U_n}{2}.$$

As was the case in regard to the formulas for obtaining the theoretical predicted means of the segregating generations, the addition of another factor pair increased the mean of the B_1 to P_1 by $\log KK+\log Kk/2$, the mean of the F_2 generation by $\log KK+2\log Kk+\log kk/4$ and the mean of the B_1 to P_2 by $\log Kk+\log kk/2$. However, before drawing conclusions the assumptions basic to the derivation of the formulas should be considered. First, it should be noted that there were no assumptions nor were any necessary concerning the relative magnitudes of KK, Kk, and kk. Hence, the formulas are applicable regardless of genic dominance (intra-allelic interactions). Likewise, there were no assumptions, nor were any necessary, regarding the relative magnitudes of K_1K_1, $K_2K_2+\cdots+K_nK_n$, nor any of their alleles. As a consequence, the formulas are applicable irrespective of the differences that may exist between the effects of the various gene pairs. Also, since KK, Kk, and kk represent all the combinations of any contrasted alleles and further since the formulas were developed on the assumption that the two parents differed by an indefinite number of gene pairs ($P_1=K_1K_1K_2K_2\cdots K_nK_n$ and $P_2=k_1k_1k_2k_2\cdots k_nk_n$), it is apparent that the formulas are applicable irrespective of the number of genes differentiating the character being studied. Finally, the matter of linkage relationships needs to be considered.

In attacking this problem consider the formula for the theoretical F_2 population, which as previously shown is: $F_2\text{ generation}=[KKU_1+KKU_2+\cdots+KKU_n]+2[KkU_1+KkU_2+\cdots+KkU_n]+[kkU_1+kkU_2+\cdots+kkU_n]$. Our problem is to determine how linkage, independent inheritance, or a combination of the two affects this formula. To begin with, it is evident that the genotypes resulting from the segregating of any factor pair (Kk) occur in the ratio of 1:2:1 regardless of linkage

relationships. That is, the ratio of n KK:n Kk:n kk is 1:2:1. But this formula regardless of the ratio of genotypes within brackets reduces to: F_2 generation = log KK + 2 log Kk + log kk/4 + log U_1 + log U_2 + ··· + log U_n/4 by applying the algebraic laws that the addition of logarithms is equivalent to the multiplication of the original figures and that addition is associative. Hence, since this is the same formula for the theoretical F_2 population as previously derived, the formulas for obtaining the theoretical predicted for the segregating generations are applicable regardless of whether the gene pairs differentiating the character under consideration are independently inherited, are linked, or some are linked and others are independently inherited. The same can be shown to be true for the formulas used in predicting the theoretical means of the B_1 to P_1 and the B_1 to P_2 populations.

The obtained and predicted means expressed in grams and the obtained and predicted means and standard errors of logarithms are given in table 4. The last three columns of table 4 furnish a method of testing whether or not the obtained means of logarithms are significantly different from those predicted by the use of WRIGHT's formulas. It will be remembered that WRIGHT's formulas are based on the assumption that the effects of the genes differentiating the character under consideration are multiplicative. The discrepancies between the obtained and predicted means listed under differences of table 4 show that in all three cases the predicted mean

TABLE 4

Obtained and predicted means expressed in grams and the obtained and predicted means and standard errors of logarithms.

PARENT OR GENERATION	MEANS EXPRESSED IN GRAMS		MEANS AND STANDARD ERRORS OF LOGARITHMS		DIFFERENCES
	OBTAINED	PREDICTED	OBTAINED	PREDICTED	
Red Currant	0.92		−0.053440±0.012217		
B_1 to Red Currant	2.10	2.29	.286961± .015886	0.327001±0.014639	0.040040±0.021602
F_1 generation	5.48		.707441± .016714		
F_2 generation	5.38	6.56	.672012± .012550	.758239± .019320	.086227± .023038
B_1 to Danmark	16.11	17.16	1.161870± .009338	1.189478± .023069	.027608± .024887
Danmark	51.17		1.671515± .028018		

is slightly greater than the obtained mean, but the following should be noted. The fit is good between the obtained and predicted means for the B_1 to Danmark; is fair for the B_1 to Red Currant, the difference being just slightly less than two times its standard error; and is poor for the F_2 generation, since the difference is somewhat more than three times its standard error. From these results it cannot be concluded that the effects of the genes are strictly geometrically cumulative. However, columns 2 and 3 of table 4 show that the discrepancies between the obtained and

predicted means are small; and because the means predicted on the hypothesis that the effects of the genes are arithmetically cumulative are 3.20, 15.76 and 28.33 grams, respectively, for the B_1 to P_1, the F_2 generation, and the B_1 to P_2, it is evident that the multiplicative hypothesis is vastly closer to actuality than is the arithmetic.

SUMMARY

The data for this study comprise weights of individual tomato fruits of the parents and progenies from an original cross of Danmark variety (*Lycopersicon esculentum* Mill.) with Red Currant variety [*L. pimpinellifolium* (Jusl.) Mill.].

A graph is given that shows the percentage of genetic variance included in the variance for between means of blocks when the number of individuals grown per block varies. The experimental design was that of a randomized block. From the graph the number of individuals necessary per block to attain a certain degree of accuracy can be determined.

The "within block" environmental variances, calculated from individual plant data, were found to be functions of the means and were found to form a geometric progression. Information as to the nature of the series of environmental variances is essential to the development of methods of separating the genetic from the environmental variation.

A method of separating that portion of the variance due to segregation of the genes from that portion of the variance due to environmental influences is presented. The method as it is extended to apply to other data should prove of considerable value in attacking the difficult problem of quantitative inheritance.

The data furnish rather convincing evidence that genic dominance occurs in the cross of Danmark × Red Currant.

As regards the nature of the interaction of the genes, the data show that the multiplicative hypothesis is vastly closer to actuality than is the arithmetic. In fact the differences are slight between the obtained means and those predicted on the basis that the effects of the genes differentiating weight of fruit are geometrically cumulative.

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THE EFFECT OF FAST NEUTRONS ON CHROMOSOMES IN MEIOSIS AND ITS BEARING UPON PACHYTENE PAIRING[1]

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PREVIOUS experiments have shown that quantitatively the response of chromosomes of *Vicia faba* in pachytene to X-rays is approximately the same as that of the same species at the onset of the somatic prophase (MARSHAK 1939a). CREIGHTON (1941) using methods similar to those employed in the Vicia studies found that meiotic chromosomes are only a sixth as sensitive as the mitotic chromosomes in Chorthippus. SAX and SWANSON (1941) have asserted that meiotic chromosomes of Tradescantia are more sensitive to X-rays than mitotic, although the meiotic stages used in their comparison are not identified.

The experiments reported here show that the somatic chromosomes of *Vicia faba* at the onset of the somatic prophase have the same sensitivity to neutrons as chromosomes in pachytene.

MATERIALS AND METHODS

In a previous report (MARSHAK 1939a) the method of identifying the meiotic stages of *V. faba* was briefly outlined, but will be described here in greater detail. In the axil of each leaf of the upper portion of the mature plant is an inflorescence bearing about six blossoms. Subsequently it was found that the stamens in each flower bud are in two sets, one longer than the other, and that all the stamens of any one set are approximately in the same stage. Since the microsporocytes of the longer set are usually more advanced in the early stages, it is necessary that comparable stamens be used in following meiotic development (table 1). In these experiments only the longer set of stamens was used. The buds were slit with a needle which had been ground to a cutting edge, and a single anther was removed. The slit was covered with a small patch of Scotch tape. The bud was then left on the plant and the inflorescence covered with moist absorbent paper and waxed paper to prevent desiccation. Buds found to have anthers with microsporocytes in pachytene were left on the plant for various time intervals, after which the entire bud was removed and fixed in a mixture of equal parts of 50 percent acetic acid and 50 percent ethyl alcohol. After fixation each of the anthers was separately smeared and stained in aceto-carmine and counts were made of the frequency of different stages.

The data on the frequency of different meiotic stages in anthers at vari-

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ous intervals after the examination of the first anther are shown in table 2. It is evident that cells found to be in anaphase I were in pachytene 22 to

TABLE 1

Distribution of meiotic stages in the different anthers of a bud. Pk = pachytene, Dk = diakinesis, Dp = diplotene, MI = first metaphase, AI = first anaphase, TI = first telophase, MII = second metaphase, AII = second anaphase, TII = second telophase, Td = tetrad. Anthers 1–5 are the long set and 6–10 are the short set.

FIRST COUNT	TIME INTERVAL IN HOURS	SECOND COUNT								
ANTHER 1		2	3	4	5	6	7	8	9	10
Pk 163	16	Dk 702	Dk 616	Dk 509	Dk 338	Dk 265	Dp 115	Dp 138	Dp 213	Dp 351
Pk 101	16	Dk 85	Dk 178	Dk 209	Dk 197	Dp 97	Dp 135	Dp 257	Dp 125	Dp 205
Pk 103	24	TI 33	TI 49	TI 196	TI 137	TI 206	TI 47	TI 197	TI 216	TI 134
		MII 81	MII 183	MII 34	MII 51		MII 82			
					AII 93					
Pk 127	24	MII 115	TI 32	MII 149	MII 57	MI 39	AI 56	AI 3	TI 168	AI 98
		TII 180	MII 269	AII 83	AII 20	TI 64	TI 175	TI 156	MII 58	TI 182
Pk 279	24	TII 54	MII 5	MI 17	TII 126	DP 6	MI 43	MI 201	MI 26	MI 44
			TII 230	AI 8	Td 135	MI 36		TI 61	TI 45	TI 9
				TII 276		AI 1				

TABLE 2

Distribution of stages at various time intervals after pachytene.

FIRST COUNT		SECOND COUNT							
TOTAL	% PACHYTENE	TIME INTERVAL IN HOURS	TOTAL	% PACHYTENE	% DIPLOTENE	% DIAKINESIS	% MI	% AI & TI	% II
1361	100.0	6, 7, 8	2822	100.0					
487	100.0	12½	1070	8.8	75.7	14.9	0.6		
990	100.0	16	699	2.7	24.4	54.9	8.3	9.6	
171	100.0	16	614		31.4	65.1	3.4		
221	100.0	17	1006		0.5	79.3	5.0	15.2	
316	100.0	17	352			96.6	3.4		
182	100.0	18	517		9.1	90.9			
239	100.0	18	510			68.8	30.4	0.8	
235	100.0	19	1284			58.9	41.1		
137	100.0	20	287			60.3	39.7		
160	100.0	21	586		2.7	0.3	64.8	32.1	
454	100.0	21	3010			3.8	80.7	15.4	
222	100.0	22	1123			28.7	40.3	27.1	3.9
56	100.0	22	462				69.0	31.0	
324	100.0	22½	389		32.9	21.1	10.3	34.5	1.3
336	100.0	23	330			1.5	35.7	62.7	
128	100.0	24	405		1.0		38.0	56.0	4.9
429	100.0	24	625			3.2	2.2	94.5	
343	100.0	24	1669				4.0	20.6	75.4
163	100.0	24½	214					55.1	44.9
166	100.0	24½	226					57.9	42.1
61	100.0	25	168						100.0

24 hours earlier. From these data one may also determine the approximate duration for pachytene, diakinesis, metaphase I, and anaphase I. In figure 1 the percentage cells in pachytene is plotted as a function of time after examination of the first anther. It appears from the graph that pachytene lasts from eight to 12 hours with a mean at ten hours. Similar graphs are shown for diplotene in figure 1, for diakinesis in figure 1 and for MI and AI plus TI in figure 2. Cells in diplotene appear with a high frequency 12–16 hours after pachytene, with a smaller percentage as late as 23 hours.

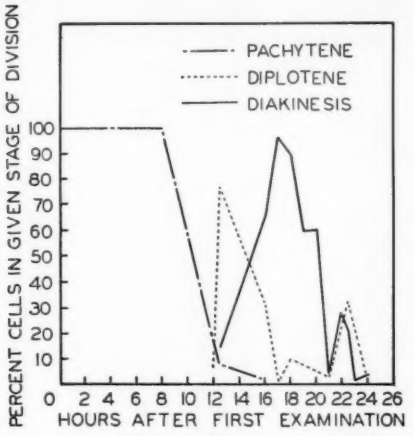

FIGURE 1.—Percentage cells in pachytene, diplotene and diakinesis as a function of time after first examination.

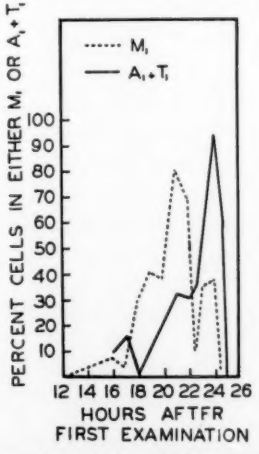

FIGURE 2.—Percentage cells in metaphase I and anaphase I as a function of time after first examination.

The average duration of the diplotene stage thus appears to be about four hours. Diakinesis reaches a maximum frequency at 17–18 hours after the first examination, while metaphase I is most frequent at 21 hours and anaphase I at 23 hours. From these curves it also appears that cells may

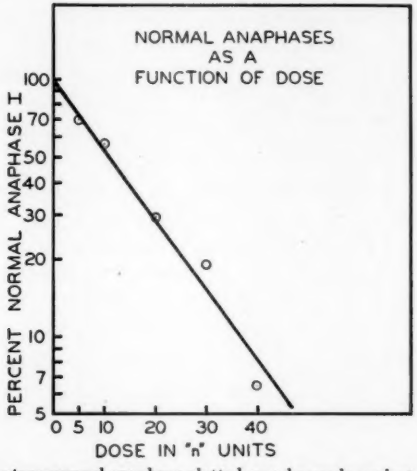

FIGURE 3.—Percentage normal anaphase plotted on a log scale against dose in neutrons.

TABLE 3

Distribution of stages following diakinesis and metaphase.

FIRST COUNT					SECOND COUNT					
TOTAL	% DIPLOTENE	% DIAKINESIS	% MI	% AI-TI	TIME INTERVAL	TOTAL	% DIAKINESIS	% MI	% AI-TI	% II
874	58.4	36.8	4.8		1	163	48.5	51.5		
53		94.3	3.8	1.9	2	375		99.2	0.8	
161		97.5	2.5		2	545	3.8	34.1	37.5	24.5
582	5.2	94.3	0.5		3	665	27.4	72.6		
185	2.7	97.3			4	565	20.7	52.4	6.8	20.1
2525		69.8	24.3	5.9	4	1261	0.2	11.7	0.4	87.8
396		23.2	76.8		1	1487	22.5	43.8	16.2	17.5
350		10.0	90.0		2	1943		44.4	43.0	12.5
945		38.6	41.4	20.0	3	981			100.0	

be in diakinesis for two to four hours, in metaphase for a similar period, and in anaphase for one to two hours. The duration of diakinesis was obtained by allowing buds whose anthers were found to be in this stage to remain on the plant and fixing such buds at hourly intervals afterwards. Data obtained in this way are given in table 3 and show, as did the graphs

described above, that diakinesis and metaphase I each last two to four hours. It may be noticed in tables 2 and 3 that some buds have a considerable percentage of the microsporocytes in the second meiotic division by 24 hours after the removal of the first anther. These plants were all older, with shoots three to four feet tall. Younger plants (one to two feet) have a more uniform distribution of stages (table 1). Similar behavior was found in Gasteria. In these and previous experiments all plants were grown in 8 inch pots in the greenhouse during the months April, May, and June and were irradiated when one to two feet tall. All plants were grown from a single lot of seed (variety Bell Windsor) obtained from a commercial seed firm.

From the data presented above it seems evident that microsporocytes found in anaphse I were in pachytene 23 to 24 hours earlier.

Conditions for irradiation of the buds with X-rays were the same as those previously described (MARSHAK 1939a). The source of neutrons used was a beryllium target bombarded by 16 million volt deutrons produced by the cyclotron. The neutron beam was collimated by a lead-lined channel embedded in a large block of paraffin (AEBERSOLD 1939). The plants were placed so that the buds to be treated were in the center of the 10×10 cm field at the end of the collimator. The neutron dose was measured in arbitrary "n" units, an "n" unit being that amount of ionization produced by neutrons which gives the same reading on a 100 r Victoreen thimble ionization chamber as does one roentgen of X-rays. We are indebted to DR. P. C. AEBERSOLD for measurements of the neutron doses.

Twenty-three to 24 hours after treatment the buds were fixed in the acetic acid-alcohol mixture and stained in acetocarmine. Only cells in mid- or late anaphase I were examined. These were classified as either normal or abnormal, an abnormal cell being one containing one or more chromosome or chromatid attachments or fragments.

The frequency and percentage of normal anaphases after doses of 5–40 n is given in table 4A. The controls showed 5 percent abnormal anaphases. Previous experiments with plants from the same lot of seed showed only 1–2 percent abnormalities in the controls for both mitosis and meiosis. The increase in abnormalities may be due to changes in growing conditions and hence may be real or may be only apparent due to changes in fixing and smearing technique introduced by the second author. In figure 3 the logarithm of the percentage normal anaphases from the corrected values is plotted as a function of the dose. The curve is of the form $Y = e^{-kx}$ as in the case of X-rays (MARSHAK 1939a). Table 5 gives the value of the slope (k) obtained by fitting the percentage normal anaphases by least squares. In fitting the curve the weight assigned to each point was the number of cells observed. Tables 4C, 4B, and 4D give the data for mitosis following neutron treatment and meiosis and mitosis following X-ray

treatment. The slopes for each of these is given in column k of table 5. In column s of the same table are given the root mean square deviations and in column R, a measure of the goodness of fit where

$$R^2 = \frac{1 - \Sigma(z - Z)^2}{n\left\{\frac{\Sigma(z^2)}{n} - \frac{(\Sigma z)^2}{n}\right\}}$$

z = observed log percentage normal anaphase
Z = calculated log percentage normal anaphase
n = number of points (YULE and KENDALL 1937)

TABLE 4

	MEIOSIS—24 HOURS AFTER IRRADIATION				MITOSIS—3 HOURS AFTER IRRADIATION			
	DOSE	NORMAL	TOTAL	% NORMAL	DOSE	NORMAL	TOTAL	% NORMAL
			A				C	
Neutrons—dose in n units.	0	1106	1163	95.0	0	1214	1228	99.0
	5	276	423	65.2	5	177	255	69.4
		79	120	65.8		985	1381	71.2
		76	120	63.3	10	189	381	49.6
		48	72	66.6		473	952	49.7
	10	290	568	51.0	20	25	121	20.6
		303	582	52.0		122	523	23.4
		181	348	52.0		138	573	24.2
		42	79	53.1	23	64	354	18.0
	20	31	115	26.9		17	95	17.9
		60	246	24.3	29	31	246	12.6
		87	357	24.3	30	21	182	11.5
	30	28	187	14.9	32	16	130	12.3
		35	251	13.9	43	2	39	5.1
		16	115	13.9	60	2	154	1.3
		45	314	14.3			D	
	40	4	166	2.4	0	1748	1777	98.4
		5	382	1.3	43	297	526	56.4
			B			383	581	65.9
X-ray—dose in r units.	0	111	112	99.1	84	128	265	48.3
	25	1145	1405	81.5		131	265	49.4
	50	1083	1877	57.8	100	120	306	39.2
	75	232	448	51.8		200	463	43.2
	100	547	1504	36.4		155	419	37.0
		243	977	24.8		174	405	43.2
	150	49	216	22.6	122	240	909	26.4
	200	98	751	13.0	125	252	1020	24.9
	250	8	170	4.7		278	1039	26.8
	300	43	842	5.1	130	130	481	27.0
						141	543	26.0
						177	640	27.6
					259	36	659	5.5
					302	7	229	3.1

The constants given have all been corrected to the base e of natural logarithms.

With X-rays, the slopes and thus the sensitivities to X-rays of chromosomes in meiosis and mitosis agree to within 2 or 3 percent. The difference in slopes is $.0003 \pm .002$ and is clearly not significant. With neutrons using the uncorrected slope the difference between meiosis and mitosis is $.010 \pm .017$, and with the corrected values $.010 \pm .008$, in both cases it is

TABLE 5

		k_e	S_e	R
Neutrons	Meiosis a*	.0814	.0168	.982
	b	.0613	.0071	.962
	Mitosis	.0714	.0033	.998
X-ray	Meiosis	.0150	.0017	.998
	Mitosis	.0102	.0013	.999

* In a no allowance is made for the 5 percent abnormal anaphases observed in the controls. The values in b are obtained from the corrected percentage normal anaphases.

not significant. Thus if there is a difference in the response of meiotic and mitotic chromosomes to neutrons it must be less than 15 percent.

DISCUSSION AND SUMMARY

Somatic chromosomes of several different species irradiated at the onset of prophase have all given n/x ratios of approximately 6 (MARSHAK 1938a). However, with chromosomes of a single species, *V. faba*, n/x varies from 6.6 to 15, depending on the stage of the mitotic cycle irradiated (MARSHAK 1938b). The close similarity of the ratios for somatic chromosomes in the onset of prophase and meiotic chromosomes in pachytene thus indicates the existence of similar functional conditions with respect to ionizing radiation or particles in both these stages which was postulated previously on other grounds (MARSHAK 1935, 1939a). It was further suggested that the condition common to the onset of the somatic prophase and to pachytene was the presence of closely approximated chromonematic surfaces during these stages of the nuclear cycle (MARSHAK 1939a, 1939b, 1942).

The lower limit of the diameter of the sensitive portion of the chromonema was obtained from calculations based on the theory of sensitive volumes. The value of the upper limit is obtained from the comparison of the relative efficiency of X-rays and neutrons and is based only on the average distance between ion pairs along a proton track and is thus a direct physical measurement in the sense that the dimension sought is compared with a known dimension—that is, the ion spacing in the proton track.

From these data and those previously published, the upper as well as lower limit of the diameter of the sensitive portion of the meiotic and mitotic chromonema is of the order of 10^{-7} cm. This may represent material within the chromonema having the dimensions and properties of small proteins—for example, histones (MARSHAK 1938a, 1938b)—or may represent the average distance between the approximated chromonematic surfaces. It has commonly been assumed that chromonemata are made up of large protein molecules. If the dimensions obtained in these experiments are referred to the chromonema, they indicate that the fundamental constituents are not necessarily chains of unusually large proteins but of some of the smallest known—that is, histones. If the dimensions are referred to inter-chromonematic spacing during pairing, then chromonemata approach to within molecular distances of each other. In either case it would seem that in seeking an explanation of pairing and crossing over, consideration should be given to molecular forces appropriate for structures of these dimensions.

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THE EFFECTS OF TRANSLOCATION ON GROWTH IN ZEA MAYS[1]

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THE concept and proof that the effect of a gene is dependent upon its location in respect to other genes as well as on its innate constitution has developed almost entirely from experiments on *Drosophila melanogaster*. Proof of "position effects" is inadequate in other organisms. The evidence from Drosophila experiments has been critically reviewed by DOBZHANSKY (1936) and MULLER (1941). This evidence has been used as the basis for theorizing on the mechanism of gene and chromosome action and has even been used as the foundation for a new theory of speciation. Additional evidence is certainly to be desired from plant material since there is almost a complete lack of critical evidence that plant genes are influenced by their spatial relationships. The experiment to be reported here was designed to detect any phenotypic changes that might result from rearranged chromosomes in an inbred line of *Zea mays*.

In the numerous reports of chromosomal rearrangements found in plants only four indicate position effects. CATCHESIDE (1939) found an X-ray-induced translocation in Oenothera which was accompanied by a reduction of pigmentation in the flower buds. Evidence that this was a position effect came from a single plant with normal pigment in a subsequent backcross generation. This plant resulted either from a crossover transferring the affected gene to a normal chromosome where it regained its normal expression or from a mutation. According to CATCHESIDE the former is the probable explanation, since a mutation of this type had never been observed, but until the results can be confirmed by a repetition of the experiment, the possibility of a mutation cannot be entirely dismissed. This evidence is the most critical obtained so far from plant material, and the technique used by CATCHESIDE gives promise of more of the same type.

BLAKESLEE and BERGNER (1940) attributed the change from rough leaves to smooth in a bud sport in Datura to a segmental interchange. The detailed evidence which would eliminate the effect from a concomitant intragenic mutation or deficiency is not given in the preliminary report.

Paired mosaics found in maize endosperm (JONES 1937, 1939a, 1940) are the result of relocated chromosome segments. The cytological basis for this

[1] This paper is part of a dissertation presented for the degree of Doctor of Philosophy in YALE UNIVERSITY.

interpretation was provided by CLARK and COPELAND (1940). Certain paired dark and colorless areas are found in which the dark area is lighter than the surrounding normal tissues. This indicates that the relocated *C* gene has been altered in its function to act as an inhibitor. The position effect hypothesis permits a satisfactory explanation of this situation, but the evidence does not necessarily eliminate alternative interpretations.

Numerous aberrant chromosome types were found in *Vicia sativa* as a result of X-ray treatment (SAVCHENKO 1935). One translocation was found that was less fertile when homozygous than heterozygous. In the absence of critical evidence, this condition can be interpreted equally well as a recessive gene mutation or deletion, or as a position effect.

In these four reports of possible position effects in plants, the Oenothera case is the only one that is supported by critical evidence. The explanation is not obvious for this almost complete lack of unquestionable cases in plants in contrast to the numerous examples in Drosophila. It is possible that the plant chromosomes have not been broken and reattached at the critical points to produce an effect, or if effects have been produced, they have been passed over unnoticed. The latter seems likely, since most of the chromosomal rearrangements reported in plants have been in heterogeneous material, and differences that might have occurred were possibly obscured by genetic segregation. Also, chromosomal rearrangements have been studied mainly from the standpoint of adding information on the mechanism of meiosis and not to determine the possible effects they might have on plant growth.

An exception to this was the experiment by BRINK (1932). Maize plants homozygous for a translocation were compared with sib plants with normal chromosomes for dry weight of plants and ears and for rate of development as measured by the number of days to silking. The differences were not statistically significant for dry weight of plants and ears. The homozygous translocated plants were 1.68 ± 0.46 days earlier than the normal, which is possibly significant statistically. However, the means in this experiment were based on about 30 plants, and as pointed out by BRINK, the high probable errors indicate the need for larger samples. A probable error based on such small numbers is itself subject to a fairly large sampling error, which casts some doubt as to the real significance of the differences found.

It seemed desirable that the rather limited study made by BRINK on the problem of position effects in plants should be extended. The present experiment was designed with this purpose in mind. The general plan was to produce chromosomal rearrangements in an inbred line of maize and to compare plants carrying these rearrangements, both in the homozygous and heterozygous condition, with the normal inbred from which they came.

EXPERIMENTAL TECHNIQUE

All of the chromosomal rearrangements in this experiment were produced in the inbred line, Connecticut 20. This is a white seeded inbred which had been produced by 12 generations of inbreeding of the Illinois High Protein strain of Burr White dent. The uniformity of C20 made it especially favorable material for the detection of small phenotypic changes, since the obscuring effects of genetic segregation had been reduced to a minimum. The only genetic factor that was found to be segregating in this line was one for a recessive yellow green plant character, and it apparently arose as a spontaneous mutation in the generation or two prior to the starting of the present experiment. Plants homozygous for this recessive character are yellowish in all stages, show poor viability, with many plants dying, and only an occasional plant sheds pollen. The classification of these plants is unmistakable. The genetic factors for plant color (EMERSON, BEADLE and FRASER 1935) that were known to be homozygous were *A b pl* r^{rr} *c Pr i*.

In 1938, pollen of the inbred line was irradiated with 1000 r units.[2] Twelve ears were pollinated with the X-rayed pollen, and from this seed, 190 F_1 plants were grown. Pollen was examined from all the F_1 plants, and the results of the classification are summarized in table 1.

TABLE 1

Pollen classification of F_1 plants following irradiation.

	NORMAL POLLEN	SEG. 50% EMPTY	SEG. ±75% EMPTY	SEG. SMALL POLLEN
No. Plants	75	49	21	45
Percentage	39.5	25.8	11.1	23.7

All the plants having abnormal pollen were self pollinated, but many of the plants failed to set an ear. STADLER (1932, 1933) has pointed out that such sterility is frequently found in X-rayed maize, since many of the induced variations are eliminated in the gametophyte generation.

Progenies were grown from all of the F_1 ears produced. Many of these progenies segregated for plants with normal and abnormal pollen in about equal numbers. The plants with abnormal pollen usually had approximately equal percentages of normal and aborted pollen grains which indicated the presence of reciprocal translocations (BRINK and BURNHAM 1929). In some of the progenies, however, the abnormal pollen bearing

[2] This dose was emitted by a Coolidge tube operating at 200 KV with unfiltered irradiation. The author is indebted to the authorities of the YALE UNIVERSITY SCHOOL OF MEDICINE for administering this irradiation.

plants varied from considerably less to considerably more than 50 percent aborted pollen. The pollen was stained with iodine and examined under a microscope.

In examining the pollen from all of the F_2 plants, it was observed that the amount of red pigment was noticeably reduced in the anthers of all plants segregating for empty pollen. The higher the percentage of empty pollen the greater was the reduction in pigment. This reduction in anther pigment is apparently a physiological response conditioned by the failure of the pollen to develop normally. It is obviously only secondarily related to genic factors or chromosome aberrations which cause the abortion. That this is true is shown by the fact that the character appeared in all progenies, and there was no segregation with progenies. The fact that all semi-sterile plants in subsequent generations, regardless of the progeny, were lighter in anther color than the normal plants is further evidence of the causal relationship between the pollen abortion and the failure of the anther pigment to develop normally. This fact was used in checking the pollen classification made with a microscope.

In order to confirm the presence of chromosomal rearrangements, all of the F_2 progenies segregating for semi-sterility were examined cytologically using the technique described by McCLINTOCK (1930). Seventeen of the progenies showed reciprocal translocations. No inversions were found, possibly because of the inadequacy of the technique used in searching for them. Anaphases were examined for bridges and fragments, and such a technique would fail to detect inversions which include the centromere.

The method described by BRINK and BURNHAM (1929) was used to identify the plants homozygous for translocations in the F_2 progenies found segregating for interchanges. The approximate 50 percent of the plants with semi-sterile pollen were heterozygous for the translocation involved and were used only for cytological material. Among the plants with normal pollen, one-half should be homozygous for the translocation and one-half homozygous normal. Since these two types were phenotypically indistinguishable, it was necessary to test all the normal plants by backcrossing to the normal C20 inbred. The homozygous translocated plants produce only plants with semi-sterile pollen in such a backcross, while the homozygous normal plants produce plants with normal pollen. Once a homozygous translocation is identified by this technique, it may be propagated indefinitely by self pollination. Thirteen of the 17 translocations were made homozygous by this method, and sufficient seed was produced to permit a rather extensive comparison with the normal C20 inbred in the following generation. Enough seed from the test backcrosses was also available for ten of the translocations so that they could be compared in the heterozygous condition.

CYTOLOGICAL IDENTIFICATION AND DESCRIPTION OF TRANSLOCATIONS

The translocations were first detected cytologically in the F_2 progenies segregating for semi-sterility. By examining the microsporocytes at diakinesis 1, the plants heterozygous for a translocation were identified by ring configurations (BURNHAM 1930; MCCLINTOCK 1930). The plants were then examined at the mid-prophase of meiosis in order to identify the chromosomes involved in the interchanges and the approximate positions where the breaks occurred. The positions of 11 of the 13 translocations were determined, and these are indicated in table 2. The system originated by ANDERSON (1938) is used for designating the points of interchange. The points are recorded in decimal fractions of the distance from the

TABLE 2

Summary of the cytologically identified translocations giving their approximate positions.

TRANSLOCATED CHROMOSOMES	PEDIGREE	APPROXIMATE POSITION
1–4	20XR-29	1S.4 4L.8
1–4	20XR-42	1L.4 4S.3
1–6	20XR-28	1S.2 6S.1
1–8	20XR-20	1L.5 8S.5
1–10	20XR-41	1L.1 10S.1
2–5	20XR-50	2S.1 5L.5
3–6	20XR-34	3S.4 6L.6
4–5	20XR-30	4L.5 5L.8
4–5	20XR-32	4S.5 5L.6
4–5	20XR1-8N	4S.6 5S.3
4–5 & 6–?	20XR1-11N	4–5 same as 20XR1-8N 6–? unidentified
1–6–8	20XR-26	1S.3 6L.6 8L.1
1?–5?–?	20XR-35	unidentified

centromere to the end of the chromosome determined by averaging the measurements from several cytological figures. The designated positions are to be considered only as close approximations in the absence of confirmatory genetical data. All the cytological examinations were made of aceto-carmine preparations.

A diagrammatic summary of the translocations that were identified is shown in figure 1. The points of breakage are indicated by the blank spaces, and opposite these points are designated the chromosomes involved in the different interchanges. For example, chromosome 1 was broken toward the distal end of the long arm and was translocated with chromosome 8 as indicated by the number opposite that breakage point. The point on chromosome 8 at which this translocation occurred is designated by the number 1. The points on chromosome 1, 6, and 8 marked by the double

figures designate the translocation between three chromosomes. Two of the translocated stocks showed exchanges between three chromosomes; one stock carried two separate translocations and the remainder were single reciprocal translocations. All of the chromosomes were involved in the translocations except numbers 7 and 9. The knob constitution of the inbred C20 is indicated in figure 1.

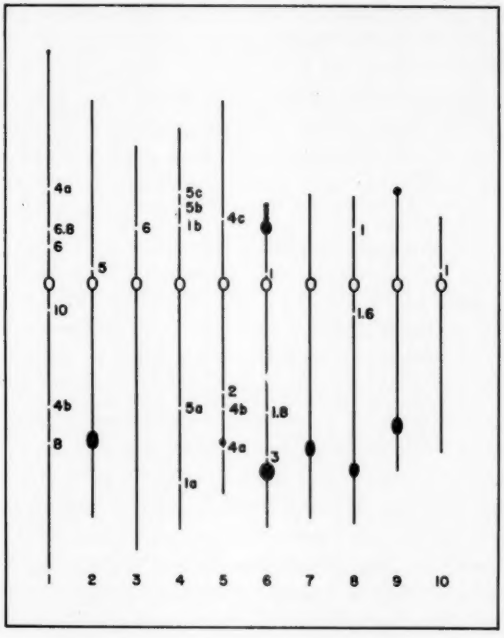

FIGURE 1.—A diagrammatic summary of the cytologically identified translocations. The knob constitution of the C20 inbred is shown.

COMPARATIVE STUDY OF THE TRANSLOCATIONS

Field test

In order to detect any changes that might have resulted from the chromosomal rearrangements, homozygous and heterozygous progenies of the translocations were grown under field conditions in comparison with the normal untreated inbred from which they came. Preliminary evidence from the preceding generation indicated that no striking differences were to be expected in any of the translocated stocks. This evidence was based on the fact that in all the progenies segregating for one-half heterozygous translocated, one-fourth homozygous translocated, and one-fourth normal

plants, no differences could be detected by observation. However, the observations were made on approximately 20 plants per progeny, and significant differences in such a limited population might easily have been obscured by soil heterogeneity or other environmental factors. It was thought that significant differences might be detected if some of the obscuring effects of the environmental factors were removed statistically. The present test was designed to permit a statistical analysis of any plant measurements that seemed desirable. To facilitate handling in a suitable field plot arrangement, the translocated stocks were divided into four groups as follows:

Group 1		*Group 3*	
1. Homo 1-8T	20XR-20	1. Homo 1-5-?T	20XR-35
2. Hetero 1-8T	20XR-20×Normal C20	2. Hetero 1-5-?T	20XR-35×Normal C20
3. Homo 1-6-8T	20XR-26	3. Homo 4-5T	20XR-32
4. Hetero 1-6-8T	20XR-26×Normal C20	4. Hetero 4-5T	20XR-32×Normal C20
5. Homo 1-6T	20XR-28	5. Homo 1-10T	20XR-41
6. Hetero 1-6T	20XR-28×Normal C20	6. Homo 1-4T	20XR-42
7. Normal C20		7. Homo 2-5T	20XR-50
		8. Normal C20	
Group 2			
1. Homo 1-4T	20XR-29	*Group 4*	
2. Hetero 1-4T	20XR-29×Normal C20	1. Homo 4-5T	20XR1-8N
3. Homo 4-5T	20XR-30	2. Hetero 4-5T	20XR1-8N×C20
4. Hetero 4-5T	20XR-30×Normal C20	3. Homo 4-5 6-?T	20XR1-11N
5. Homo 3-6T	20XR-34	4. Hetero 4-5 6-?T	20XR1-11N×C20
6. Hetero 3-6T	20XR-34×Normal C20	5. Normal C20	
7. Normal C20			

Group 1 was arranged in two 7×7 Latin squares which were separately randomized and placed side by side in the field. This permitted 14 replications for each entry in single row plots 12 feet long. Groups 2 and 3 were handled in the same way as Group 1. In Group 3, the homozygous translocation 4–5, 20XR-32, was included as an extra entry in one of the Latin squares, and was analyzed in a separate paired experiment with the Normal C20 inbred. The fourth group was randomized in a single 5×5 Latin square with single row plots 18 feet long. Seeds were planted singly nine inches apart within each plot in all of the tests so that thinning was unnecessary. Unfavorable conditions at the time of germination caused considerably more variation in germination than was anticipated. The extent of the variation in number of plants between entries can be observed in tables 3, 4, 5, and 6.

All plots were maintained under as nearly uniform cultural conditions as possible and were kept under close observation throughout the growing season. No striking differences appeared. This confirmed the limited observations made in the preceding generation. Even though no phenotypic changes could be observed in the field, it was thought that small signifi-

cant differences might exist which would be too small to detect by observation. For this reason, individual plant measurements were made of the following characters: (1) date of pollen shedding, (2) date of silking, (3) height of plant, (4) diameter of stalk, (5) width of leaf, and (6) length of leaf.

The date that every plant first shed pollen and showed its first silk was recorded throughout the flowering season. This was taken as a measure of rate of development. Individual plant measurements were made for the other characters after the plants had reached maturity. The diameter of stalk was measured at the mid-point of the first internode above the ground, and the length of one leaf of each plant was measured from the ligule to the tip. The leaf selected for measurement was the one arising from the ear-bearing node, since this position was found to be very constant in all progenies. The width of the same leaf was determined at the mid-point in its length.

The total variability in the double 7×7 Latin squares was segregated by an analysis of variance into the following parts: (1) between squares, (2) between varieties, (3) interaction of varieties by squares, (4) between plots within varieties, and (5) between plants within plots. In the single 5×5 Latin square, of course, the first and third sources of variability did not occur. The variance for "plots within varieties" was used as the error term for comparing the mean values of the characters measured. Included in this error term is the variability between rows and columns which ordinarily would be removed in an analysis of a Latin square. This procedure was prompted in the present experiment by the non-orthogonal nature of the tests due to the variation in stand. The experimental error was found not be unduly inflated by combining the variability of rows and columns when compared with the variance for "plants within plots." This indicates that the effect of soil heterogeneity was minor, and combining that effect with the experimental error would only result in a more conservative comparison of the means.

The results of the four tests are summarized in tables 3, 4, 5, and 6. The means for the different characters measured are shown for the homozygous and heterozygous translocations and the normal inbred C20 used for comparison. The number of plants on which the means are based and the experimental errors expressed as standard deviations are also given in these tables. On the basis of this information, the means of the translocations were compared with those of the Normal C20 by the use of the "t" test (FISHER 1934).

$$t = \frac{\text{mean difference}}{\text{stand. error of the mean difference}}.$$

Standard error of a mean difference = standard deviation $\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$,

in which N_1 and N_2 are the numbers on which the respective means are based. In order to determine its probability, the t value thus calculated was compared with the value in the t table corresponding to the number of degrees of freedom involved. As an additional safeguard, the t value has been based on 26 degrees of freedom, since each mean was calculated from 14 replications. In the single 5×5 Latin square test where each mean was calculated from five replications, there were eight degrees of freedom. When the probability of the t value was .01 or less, the mean difference was considered significant. For example, in table 2 the mean difference between the homozygous translocation 1-6-8, 20XR-26, and Normal C20 for the date of first pollen is 2.14 days. The number of plants on which the means were based is 185 and 157, respectively, and the standard deviation is 5.22.

$$t = \frac{2.14}{5.22\sqrt{\frac{1}{185} + \frac{1}{157}}} = 3.782.$$

The probability of a t value of 3.782 for 26 degrees of freedom is less than .01, so the mean difference in this case is considered statistically significant.

The means of the translocations that differed significantly from those of the normal inbred on the .01 probability level are marked by asterisks in tables 3, 4, 5, and 6. The .01 level of probability was used instead of the .05, since added assurance was desired that conclusions drawn from such small differences would be statistically valid. Each translocation should be separately inspected to see the exact magnitude and distribution of the significant differences that were found.

1-8T 20XR-20. This translocation in the homozygous and heterozygous condition was later in silking than Normal C20. The same tendency was shown in date of pollen, but the differences were not sufficiently large to be significant. A direct relationship exists between date of pollen and silks, but the correlation is not perfect, which explains the fact that a significant difference may be found for one and not the other. A significant reduction in leaf width was found in the homozygous but not in the heterozygous progeny. The other characters measured were not significantly different in either the homozygous or heterozygous translocated progenies.

1-6-8T 20XR-26. The homozygous progeny showed a significant increase over the normal inbred for two of the characters and a decrease for two others. It averaged two days earlier in shedding pollen and had a larger

TABLE 3

A comparison of three translocations in the homozygous and heterozygous condition with the Normal C20 inbred from which they were derived.

	1–8T 20XR-20		1–6–8T 20XR-26		1-6T 20XR-28		NORMAL C20	STAND. DEV.
	HOMO	HETERO	HOMO	HETERO	HOMO	HETERO		
Date of first pollen in August	16.76	16.21	13.40*	15.07	16.26	17.02	15.54	5.22
Date of first silk in August	25.47*	24.24*	21.32	23.89	23.32	24.05	22.27	5.04
Height of plant in inches	63.17	63.67	63.06	61.19	59.17*	59.18*	63.10	6.18
Diameter of stalk in cm	2.94	3.01	3.02*	3.04*	2.98	2.96	2.93	.32
Width of leaf in inches	3.70*	3.77	3.73*	3.77	3.86	3.85	3.81	.20
Length of leaf in inches	36.07	35.98	34.89*	34.12*	34.23*	34.31*	35.84	2.40
No. of plants measured	116	144	185	129	65	95	157	

* Significantly different from Normal C20 on the .01 probability level.

stalk diameter. It was thought from observation that this translocation was taller than normal throughout the growing season, but this did not prove to be the case as shown by the height measurements. The height difference that was observed during the growing season was evidently a manifestation of earliness as shown by the date of flowering and the fact that the difference in height did not exist when maximum growth was completed. The length and width of leaf were significantly less than Normal C20. The larger stalk diameter and shorter leaf were also manifested in the heterozygous progeny, but the other characters measured were not different from the normal inbred.

1-6T 20XR-26. Both the homozygous and heterozygous progenies of

TABLE 4

A comparison of three translocations in the homozygous and heterozygous condition with the Normal C20 inbred from which they were derived.

	1–4T 20XR-29		4–5T 20XR-30		3–6T 20XR-34		NORMAL C20	STAND. DEV.
	HOMO	HETERO	HOMO	HETERO	HOMO	HETERO		
Date of first pollen in August	14.92	13.59	15.91*	17.66*	16.80*	14.02	14.42	4.34
Date of first silk in August	21.63	21.72	24.59*	25.59*	22.55*	21.67	21.30	3.93
Height of plant in inches	60.72*	61.81*	63.47	59.51*	61.27*	63.34	64.21	4.98
Diameter of stalk in cm.	3.05	3.16*	3.16*	3.01	2.91*	3.08	3.04	.28
Width of leaf in inches	3.76*	3.88	3.92	3.82	3.78*	3.81*	3.88	.20
Length of leaf in inches	34.69*	34.99*	33.98*	34.42*	35.07*	35.28	35.72	2.04
No. of plants measured	60	107	96	35	154	165	192	

* Significantly different from Normal C20 on the .01 probability level.

this translocation were reduced in height and length of leaf in comparison to the normal. None of the other characters was significantly different.

1-4T 20XR-29. The height of plant and length of leaf were significantly less in both the homozygous and heterozygous progenies than in the normal. In addition, the homozygous progeny showed a reduction in width of leaf and the heterozygous progeny had a significantly larger stalk diameter.

4-5T 20XR-30. In both the homozygous and heterozygous translocations the dates of pollen and silks were later and the length of leaf was significantly reduced. The homozygous progeny showed an increased stalk diameter that did not exist in the heterozygous progeny, and the progeny heterozygous for the translocation was reduced in plant height.

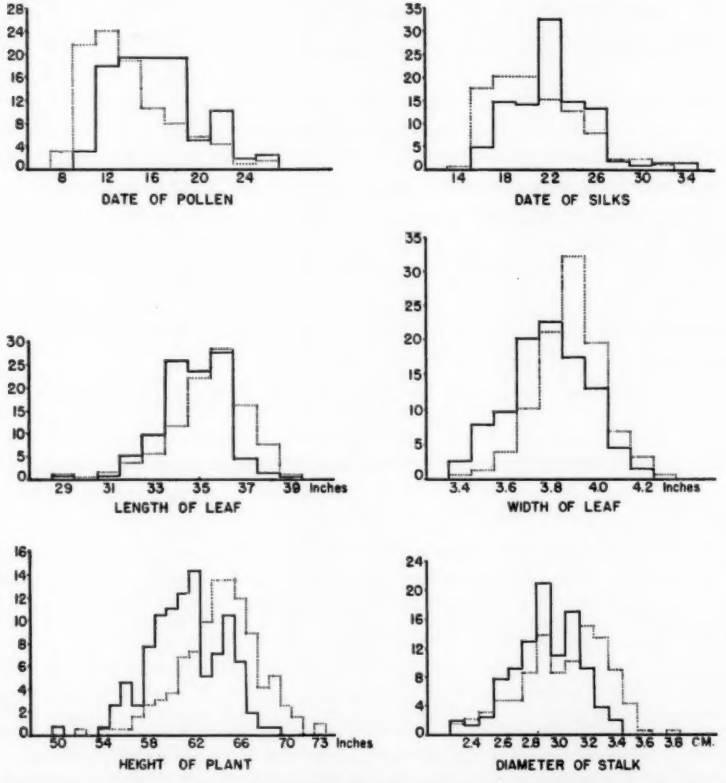

FIGURE 2.—Comparison of the homozygous 3-6 translocation, 20XR-34 (solid line), and the Normal C20 inbred (broken line). The number of plants (in percent) is shown on the ordinate of each histogram.

3-6T 20XR-34. In the homozygous condition this translocation showed a significant reduction for all of the characters measured. This is illustrated in figure 2 where the frequency distribution of the homozygous translocated plants can be compared with the normal inbred. The heterozygous progeny was not different from normal except for a reduced width of leaf.

1-5-?T 20XR-35 .The height of plant was reduced in the homozygous and heterozygous progenies, and the length of leaf was significantly less in the homozygous translocation.

TABLE 5

Five translocations compared with Normal C20. Three of the translocations were grown only in the homozygous condition.

	1–5–?T 20XR-35		4–5T 20XR-32		1–10T 20XR-41	1–4T –42	2–5T –50	NORMAL C20	STAND. DEV.
	HOMO	HETERO	HOMO†	HETERO	HOMO	HOMO	HOMO		
Date of first pollen in August	17.50	16.16	18.05	16.05	13.81	14.77	16.73	15.39	7.08
Date of first silk in August	23.35	22.43	23.86	22.91	20.33*	22.71	22.11	22.48	5.74
Height of plant in inches	58.51*	58.79*	55.55*	59.69*	58.88*	58.75*	58.30*	62.25	5.74
Diameter of stalk in cm.	2.99	3.07	2.95	3.10	2.94	3.02	3.04	3.02	.38
Width of leaf in inches	3.78	3.74	3.77	3.86	3.78	3.78	3.85	3.82	.23
Length of leaf in inches	33.04*	34.56	34.68	34.26*	33.48*	33.19*	33.61*	34.95	1.67
No. of plants measured	66	87	20	114	116	77	80	185	

* Significantly different from Normal C20 on the .01 probability level.

† Means of the homozygous progeny were compared with Normal C20 in a separate paired experiment.

TABLE 6

Homozygous and heterozygous progenies of two translocations compared with Normal C20.

	4–5T 20XR1-8N		4–5 & 6–?T 20XR1-11N		NORMAL C20	STAND. DEV.
	HOMO	HETERO	HOMO	HETERO		
Date of first pollen in August	15.38*	12.13	13.63	13.31	12.68	2.90
Date of first silk in August	22.88*	20.23	21.23	21.40	20.12	3.39
Height of plant in inches	62.19	64.59	62.20	63.20	64.97	5.95
Diameter of stalk in cm	3.10	3.09	3.02	2.97	3.05	.44
Width of leaf in inches	3.85	3.83	3.89	3.83	3.83	.18
Length of leaf in inches	34.58	35.84	34.07	35.08	35.41	3.20
No. of plants measured	26	75	60	62	59	

* Significantly different from Normal C20 on the .01 probability level.

4-5T 20XR-32. The homozygous and heterozygous progenies were significantly reduced in height, and the heterozygous progeny had a shorter length of leaf.

1-10T 20XR-41. This translocation was grown only in the homozygous condition. It was significantly earlier in silking but was reduced in height and length of leaf.

1-4T 20XR-42. The homozygous progeny of this translocation was reduced in height and length of leaf. A heterozygous progeny was not grown.

2-5T 20XR-50. The height of plant and length of leaf were also reduced in the homozygous progeny of this translocation. It was not grown in the heterozygous condition.

4-5T 20XR1-8N. The homozygous progeny was later for both date of pollen and silks than Normal C20. This was the only significant difference in either the homozygous or heterozygous progeny.

4-5&6-?T 20XR1-11N. This translocation showed no significant change from normal in either the homozygous or heterozygous condition.

DISCUSSION

In a total of 138 comparisons made between the homozygous and heterozygous progenies of the translocations and the Normal C20 inbred used as a check, 49 statistically significant differences were found. Forty-three of the 49 differences were in the minus direction from normal and six were in the plus direction. (A later date for pollen and silks and a decrease in the means for the other characters measured is considered a change in the minus direction from normal.) This indicates the general tendency for the translocated progenies to be less vigorous than normal when significant changes did occur.

The distribution of the significant differences might be of some aid in interpreting the possible causes for the differences. The frequency of the significant differences for the characters measured, and the manner in which the homozygous and heterozygous progenies were affected in relation to each other is shown in table 7. For example, the differences that appeared in both the homozygous and heterozygous progenies are tabulated in row 1, those that appeared only in the homozygous progenies in row 2, and those that appeared only in the heterozygous progenies in row

TABLE 7

Distribution of the significant differences showing the manner in which the homozygous and heterozygous progenies were affected in relation to each other.

CHANGE FROM NORMAL C20	DATE POLLEN		DATE SILKS		HEIGHT PLANT		DIAMETER STALK		WIDTH LEAF		LENGTH LEAF		TOTAL CHANGES	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Homozygote & heterozygote		2		4		8	2			2		8	2	24
Homozygote only	1	2		2		1	1	1		3		2	2	11
Heterozygote only						1	1					1	1	2
Total		5		6		10		5		5		11		42

3. Forty-two of the 49 significant differences found are accounted for in this table. The remaining seven appeared in the translocations that were grown only in the homozygous condition.

The fact that one-half of all the differences found are in row 1 seems a point of significance. This means that in 13 cases in which the homozygous translocations were different from normal, the heterozygous progenies were similarly affected. Such a distribution does not appear to be a random one. This evidence would seem to indicate that whatever caused the changes in the homozygous translocated progenies was also responsible for the corresponding changes in the heterozygous progenies. There can be only speculation as to the nature of the mechanism that might be responsible for these changes. The minus changes cannot be explained as recessive mutations since they also appeared in the heterozygous progenies. There were 13 significant changes in the homozygous progenies that did not appear in the corresponding heterozygous progenies (row 2). This absence of an effect suggests recessive mutations that are masked in the heterozygous condition. The possibility exists, however, that the numbers were not sufficiently large to establish the significance of the effect in the heterozygote especially if the heterozygous effect is smaller than the homozygous one.

These 26 cases involve 39 of the 49 significant changes. The three changes that appeared only in the heterozygote may possibly be position effects of the cubitus interruptus type (DUBININ and SIDOROFF 1934), but such speculation must be withheld until more evidence is obtained on these or similar cases. The remaining seven significant changes are accounted for by the differences that occurred in the translocations that were grown only in the homozygous condition.

When the means of the different progenies in which significant deviations from the normal had occurred were averaged according to the characters affected, it was found that the average is intermediate in the heterozygote between that of the normal and homozygote for each character measured

TABLE 8

Summary of the data from the different progenies in which significant changes appeared. The values given are averages of the means.

	HOMO TRANSLOCATION	HETERO TRANSLOCATION	NORMAL C20
Date of pollen	15.37	14.72	14.27
Date of silks	23.72	22.93	21.25
Height of plant	59.78	60.39	63.37
Diameter of stalk	3.04	3.07	3.01
Width of leaf	3.74	3.81	3.84
Length of leaf	34.37	34.56	35.53

with the exception of diameter of stalk, in which the means are essentially the same (Table 8).

The averaging of the data from different progenies indicates that whereas the changes involved are considerably more noticeable in the homozygous than heterozygous condition, the heterozygote is consistently intermediate between the homozygote and normal. Such a position indicates that small genetic changes produced directly by the X-rays, especially recessive mutations, are not the sole cause of the changes, but that some of the differences found are probably the result of the translocations.

The translocated progenies tested were each planted from single ears, and the question naturally arises as to whether the differences found are due to slight segregation within the inbred line. JONES (1939b) found that two sib lines were significantly different when separated from a single inbred line after 17 generations of inbreeding. This indicates the possibility that the small changes found to be statistically significant in the translocated stocks might be expected if the same number of single ear lines with normal chromosomes were similarly tested. While this possibility cannot be entirely eliminated, there is some evidence that argues against it. In one of the tests (table 3) the normal inbred progeny came from a single ear, while in the other three tests (tables 4, 5, and 6) the normal progeny came from a composite sample of eight selfed ears. With appreciable segregation in the normal inbred line, the progeny from the eight bulked ears would be expected to show more variability than the single ear progeny. Actually, there was very close agreement between the two progenies for all characters measured when they were compared statistically.

In a total of 138 comparisons made between the translocations, both in the homozygous and heterozygous condition, and the normal inbred, 49 were significantly different on the .01 probability level. Of these 49 significant differences, 43 were in the minus and 7 were in the plus direction from the normal inbred. This skewed distribution can hardly be expected from a random fluctuation around the norm, especially since the normal progeny for most of the test did not come from a single ear but from a composite sample of several ears. This evidence also indicates that the significant differences found are not to be explained by segregation between sib lines within an inbred line.

In the field experiment, the only measure of the rate of development was the time required for the plants to reach the flowering stage. The inadequacy of the criterion was fully realized, but a more exacting measure was physically impracticable under field conditions. In order to gain more exact information as to the relative growth rates of the translocated progenies and the Normal inbred, a test was conducted to compare the dry

weights of plant material produced over a period of about seven weeks under greenhouse conditions. The plants were grown in sand uniformly supplied with an inorganic nutrient solution. There is no evidence from the greenhouse test to indicate that the growth rate of Normal C20 inbred has been altered by the chromosomal rearrangements, at least during the period of 40 days after planting. The significant differences found in the field evidently occurred sometime later than the growth period tested in the greenhouse, which represented approximately the initial one-third of the life span of the inbred.

In the 13 homozygous translocations, ten of which were also tested in the heterozygous condition, no obvious changes appeared. This negative evidence is of value only in that it adds to the increasing amount of other negative evidence to indicate the rarity or even lack of marked position effect in plants. A complete summary of the cases of chromosomal rearrangements in plants which failed to produce a phenotypic effect is unnecessary to emphasize the relative scarcity of positive evidence, which was earlier reviewed in this paper. There have been more than fifty translocations reported in maize, none of which show evidence of position effect, and this number would undoubtedly be doubled by the addition of unpublished translocations that are known. Likewise, the inversions known in maize, though fewer in number than the translocations, produce no conspicuous phenotypic effect.

In Datura, BERGNER, SATINA, and BLAKESLEE (1933) have reported well over a hundred "prime types," races with chromosomal interchanges, most of which resulted from irradiation with a few obtained from nature. It was stated (p. 111) that "plants with prime types may or may not be morphologically distinct. Most of them are not and hence have been called cryptic types. However, a few obtained from radiation treatment are distinct, possibly in some cases because of excess chromosomal material present, or possibly in other cases because of genes, if such they are, that are closely linked with the modified chromosomes." This last possibility suggests position effect, but in the absence of a positive statement supported by critical evidence, it cannot be seriously considered.

The known chromosomal rearrangements without mutational effect in Oenothera, Tradescantia, Vicia, Crepis, and other plant genera added to those in maize and Datura emphasize further the extreme rarity of position effects in plants, if, indeed, they occur at all. When the number of chromosomal rearrangements in plants is considered, it would seem that ample opportunity has been afforded for the breakage and reattachments at critical points to produce visible effects. It is possible that phenotypic alterations have accompanied some of these chromosomal rearrangements, but they have been passed over unnoticed, especially since most of the re-

arrangements have been in heterogeneous material where genetic segregation may have an obscuring effect. Also, the study of chromosomal rearrangements has been concerned chiefly with gaining information on the mechanism of meiosis and more rarely with the effect on plant growth. It is not likely, however, that position effects of the same order as Bar, hairy, cubitus interruptus, mottled, etc., in Drosophila would have gone unnoticed in plants even in the most heterogeneous material. The evidence suggests that there is some fundamental difference between Drosophila and plants that more readily permits the expression of position effect in one than in the other.

An explanation of this difference at the present time must be almost entirely speculative. It has been suggested (DOBZHANSKY 1936) that the translocations in plants represent a selected group since any chromosomal aberration having a lethal or sub-lethal effect will tend to be eliminated in the haploid generation. STADLER (1941) states that "presumably if mutant effects occur at translocation points in maize the resulting mutations are eliminated in the gametophyte generation." However, no gametophyte effects were found associated with translocation when STADLER used special methods of detection. This negative evidence suggests that the basic cause of the rarity or absence of position effect in plants is not to be found in the gametophyte generation. At most, only the potential position effects of a haplo-lethal nature would be eliminated in the gametophyte generation. If position effects are to have a significant biological role in plants, at least a small percentage of them must be transmitted, and at present there is almost a complete lack of convincing evidence that any have been transmitted.

MULLER (1941) has suggested that the intensity of the synaptic forces in the somatic cells may be related to the expression of position effect. In organisms having relatively intense synaptic forces in somatic cells, position effects might be more strongly expressed, and MULLER states that "this would agree with the finding of position effect in Diptera and their absence or paucity in maize and in mice." This explanation is admittedly largely speculative.

The relatively high content of heterochromatin of Drosophila chromosomes in comparison to maize chromosomes might possibly account, at least to some extent, for the differential expression of position effect in the two. The variegated dominant eye mutants in Drosophila have been explained as position effects, and it was thought by SCHULTZ (1936) that this mosaicism always accompanied the transfer of the affected gene into the proximity of a heterochromatic region. SCHULTZ and CASPERSSON (1939) and SCHULTZ (1941) have expressed the opinion that this variegation is related to the nucleic acid metabolism in the heterochromatin, but the evi-

dence for this is at present conflicting (COLE and SUTTON 1941). GRIFFEN and STONE (1940) found evidence that mottling is not necessarily dependent on the close association of the affected gene and heterochromatin, and more evidence is certainly to be desired before extending the speculation on the effect of heterochromatin on the functioning of a gene. The role of heterochromatin in maize is being studied by ROMAN (1942) in translocations between the supernumerary "B" chromosomes, consisting largely of heterochromatic material and the ordinary "A" chromosomes. There is no evidence that any of the genes in the "A" chromosomse are altered by the new associations with the heterochromatin of the "B" type chromosomes.

Statistically significant small differences were found in the present experiment by a comparison of several plant characters in the translocated stocks and the normal inbred. These differences were purposely referred to as plus and minus changes instead of using the ambiguous and highly controversial terms, "progressive" and "retrogressive" changes. The interrelationships of the different characters measured is unknown, so it is obviously difficult to attempt to interpret the survival value or evolutionary significance of the changes.

Since the small significant differences are not likely to be the result of slight segregation within the inbred line, as was earlier pointed out, the question naturally arises as to whether they are position effects. Some of the changes are probably the result of intragenic alteration or invisible losses either associated with or independent of the rearrangements. This is indicated by the 13 instances in which the homozygous translocated progenies were different from normal and the heterozygous progenies showed no change. As was earlier pointed out, however, the intermediate position of the heterozygote between that of the homozygote and normal when the data from different progenies are averaged indicates that recessive mutations are not the sole cause of changes even in this category. In addition, there were 12 cases in which the minus differences appeared in both the homozygous and heterozygous translocated progenies indicating that factors other than recessive mutations are responsible. A complete interpretation of these small significant differences must await further experimental evidence.

SUMMARY

Thirteen reciprocal translocations were cytologically identified in an inbred line of *Zea mays* following pollen irradiation with 1000 r units. All chromosomes were involved in the interchanges except chromosomes 7 and 9. Two of the translocated stocks showed exchanges between three chromosomes; one stock carried two separate translocations, and the remainder were single reciprocal translocations. No inversions were found.

Homozygous and heterozygous progenies of the 13 translocations were compared in a replicated field test with the normal untreated inbred from which they were derived. No conspicuous phenotypic changes could be detected in any of the translocation progenies. Significant small differences, although relatively infrequent, were found by a statistical study of several plant characters. These differences were mostly in the negative direction from the normal inbred, but a few were found in the positive direction. At present there is no positive evidence that would permit these small differences, when considered separately, to be interpreted as position effects, since the possibility of an accompanying intragenic alteration or small deficiency cannot be eliminated. However, when all the evidence is considered, recessive mutations do not adequately account for all the changes found.

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THE EFFECT OF THE GENE ABDOMEN ROTATUM ON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER

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INTRODUCTION

RECENTLY considerable emphasis in genetic research has been centered on the effects of genes upon development. The determination of the time at which a gene begins to act and of the manner in which it expresses its specific action in terms of cell or tissue structure has led to a better understanding of the mechanism of gene activity. This is especially true of carefully controlled mutant stocks of *Drosophila melanogaster*, about which investigations have already yielded much important information.

A number of gene mutations in *Drosophila melanogaster* causing abdominal rotation have been reported in the literature from time to time, but no record of the embryological picture of their effects has appeared. BRIDGES and MORGAN (1923) described a mutant showing abdominal rotation which was in all cases to the left, through 60 to 90 degrees. This mutation was determined to be a simple recessive and was located in chromosome III. Aside from the counter-clockwise rotation of the abdomen in the adult, no structural changes were observed. This stock has been lost, due both to poor viability and to difficulty in mating. In 1928 DEMEREC (1938) found a mutant, twisted, located on chromosome I. The abdomen was twisted through 30 degrees in a manner similar to the first described mutant. In 1932 MOHR (1938) discovered a similar mutation located on the same chromosome as DEMEREC's twisted. The second case showed more extreme twisting than the first. A third somewhat similar mutant was also described by MOHR, but it is questionable if it was any different from the second "twisted" described.

BELIAJEFF (1931) found and named the mutation abdomen rotatum which was used in this study. He determined it to be a recessive located on chromosome IV. The abdominal rotation is clockwise, generally through 45 to 60 degrees (fig. 4). The mutant was first observed during an investigation of wild populations at the INSTITUTE FOR EXPERIMENTAL BIOLOGY at Moscow. BELIAJEFF stated that the twisting was first observed as the development of the flies neared the end, the pupal abdomen taking on an abnormal position within the puparium. A free space was found to appear in the right side of the puparium, the pupal abdomen being pressed against

the left wall. No asymmetry or abnormality of the larvae or puparia was seen. BRIDGES (1935) described still another mutant showing abdominal rotation, later determined to be an allele of the abdomen rotatum of BELIAJEFF. The rotation was similar in direction to BELIAJEFF's, but reversed its direction as the adult flies aged.

With the exception of this brief account by BELIAJEFF, investigators have described the effects of these genes in terms of external adult structures only. For this reason, the present investigation was undertaken to determine the effects of the gene abdomen rotatum on stages earlier than the adult. Special attention was also given to the position and condition of the internal organs in histological preparations.

MATERIALS AND METHODS

The stock used was *ar/ey*^{D}, a balanced lethal of abdomen rotatum with eyeless dominant. It was secured through the courtesy of DR. M. DEMEREC of the CARNEGIE INSTITUTION OF WASHINGTON, Cold Spring Harbor, N. Y. Each generation of this stock theoretically should have one fly out of three with the rotation. This proportion was cut down considerably by the apparently poor viability of the developing mutants. Several attempts were made to breed a pure *ar/ar* stock, but all except one failed. The few offspring obtained in this instance were not sufficient for use in the study. The rotated females are fertile, but matings involving the males[1] are usually sterile, presumably because of the distortion of the male copulatory organs (BELIAJEFF 1931). Crosses of rotated females with known heterozygous males produced rotated cases in approximately fifty percent of the offpsring. Repeated crosses of this type furnished most of the material studied. The Oregon R stock from the WASHINGTON SQUARE COLLEGE laboratories provided control material for comparison and contrast.

Living material was examined under a binocular dissecting microscope, both with direct light on a black background and with transmitted light. The cultures were kept at a temperature of $25 \pm 2°$ centigrade. Individuals being observed during metamorphosis were kept on microscope slides in moist chambers during the observation periods. Numerous photographs were taken as checks upon recorded observations.

For histological study, fixation in Carnoy's strong fluid or Carnoy-Lebrun was employed, with subsequent treatment according to the usual paraffin method. Sections were cut from six to ten micra in thickness and stained with Delafield's or Heidenhain's hematoxylin. In sectioning pupae, it was found that removal of the puparium by dissection prior to infiltration resulted in better sections. Some total mounts of larvae, prepupae,

[1] Dissections of adult males in a solution of one-third sea water to two-thirds distilled water (HOWLAND 1932) showed the vasa efferentia filled with actively moving sperm.

and pupae were made. Staining by both the Feulgen reaction and borax-carmine was employed. These total mounts, however, showed little that could not be seen by direct observation of living material. For study of puparium markings, fragments of puparia were washed in alcohol, cleared in xylene, and mounted in gum damar.

The prepupal stage referred to frequently in this paper includes the 11½ hour period between puparium formation and the termination of pupation movements (ROBERTSON 1936).

OBSERVATIONS

Larvae

Observations of larvae confirm BELIAJEFF'S earlier findings as to the lack of any asymmetry at this stage. Study of larvae was limited to living material from *ar/ey*^{D} stock matings and backcrosses. As yet no method has been found for separating normal larvae from larvae destined to become rotated adults. Prior to the time of puparium formation, normal and genetically rotated individuals are indistinguishable.

EXPLANATION OF PLATE

FIGURE 1.—Genetically "rotated" prepupa with gas bubble (bu). Note persistent segmentation (p.s.) of puparium.

FIGURE 2.—Rotated pupa. Note asymmetrical position of pupal abdomen within symmetrical puparium.

FIGURE 3.—Empty puparium of a rotated individual. Note segmentation of puparium (p.s.) and marked posterior constriction (p.c.).

FIGURE 4.—Rotated adult male.

FIGURE 5.—Normal prepupa prior to formation of gas bubble. Note smooth contour of puparium as contrasted with that of (1).

FIGURE 6.—Normal pupa.

FIGURE 7.—Empty puparium of normal individual.

FIGURE 8.—Normal adult male.

FIGURE 9.—Cross section of normal prepupa through genital disc (g) region.

FIGURE 10.—Cross section through genital disc (g) region of genetically "rotated" prepupa. Note symmetrical character of lateral bulges (b) in puparium. Genital disc (g) and tracheae (t) show no rotation.

FIGURE 11.—Cross section of genetically "rotated" prepupa through abdominal region, anterior to genital disc.

FIGURE 12.—Cross section through testis region of normal male pupa.

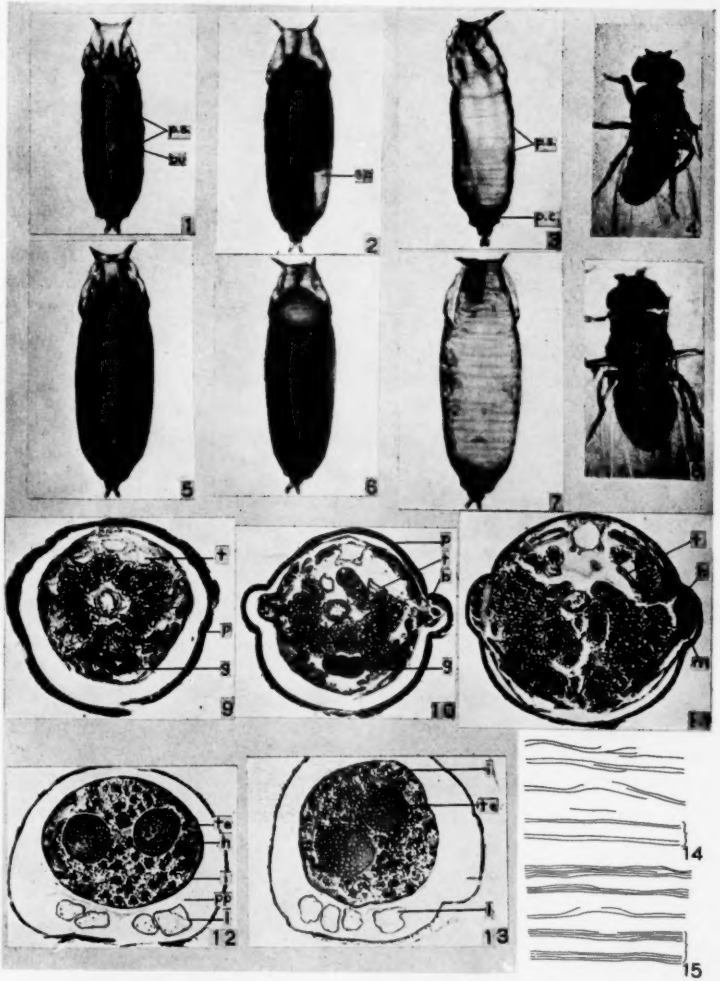
FIGURE 13. Cross section through testis region of rotated pupa. Note rotation of testes (te) and imaginal discs (i) of abdominal hypodermis.

FIGURE 14.—Drawing of a section of the dorsal surface of a normal puparium. Markings shown run transversely.

FIGURE 15.—Similar drawing of the puparium of a rotated fly.

ABBREVIATIONS USED

b, abnormal bulge in lateral wall of puparium. bu, prepupal gas bubble. h, abdominal hypodermis. i, imaginal disc of abdominal hypodermis. l, leg. m, lateral dorso-ventral muscle. p, puparium. p.c., abnormal posterior constriction of puparium. p.s., abnormal persistent segmentation of puparium. pp, prepupal cuticle. sp, abnormal space between rotated pupal abdomen and right wall of puparium. t, trachea. te, testis.



PLATE

Particular attention was given to the position of the genital disc in third instar larvae. ROBERTSON (1936) states that at the time of puparium formation this disc is the only imaginal disc in the abdominal region. It lies in the midline of the ventral surface, just anterior to the anus, and is readily seen in living third instar larvae. No deviation of this disc was observed in the material examined, although the stock was known to contain both normal and "rotated" individuals.

Puparia

The termination of the third larval instar in the normal fly is characterized by a shortening of the larva and an increase in its diameter as the larval cuticle is transformed into a puparium (ROBERTSON 1936). At this stage appears the first character on the basis of which an accurate separation of normal from rotated forms is possible. In normal individuals, the segmental constrictions of the larvae are lost, and the purparium contour becomes quite smooth (fig. 5 and 7). In individuals known to be genetically rotated, marked segmental constrictions remain after the puparium has completely hardened (fig. 1 and 3, p.s.). These constrictions are consistently present and are identified readily as soon as complete quiescence of the larva has occurred.

A second puparium abnormality is a deeply marked constriction near the posterior end just anterior to the spiracles (fig. 3, p.c.). There is a slight constriction in the normal puparium, but the exaggeration of this in the rotated cases is most striking and provides a reliable criterion for distinguishing normal from rotated individuals, prior to the time of actual rotation.

A third variation from the normal condition is to be noted in the puparial segments just ahead of the posterior constriction. Histological preparations show that here the lateral walls bulge out between the attachments of the broad lateral muscles which run dorsoventrally (fig. 10 and 11, b). This bulge apparently develops prior to complete hardening of the puparium, and sections through young individuals fixed shortly after purparium formation show the muscle attachments coinciding with the edges of the bulge. This irregularity of the lateral puparial wall, while very slight in normal individuals, is greatly accentuated in those cases which will become rotated.

Minute transverse ridges, showing a distinct segmental arrangement, first become visible when the puparium hardens. These are best seen when the animal is viewed from the dorsal aspect. The character of these ridges is distinctly abnormal in the puparia of "rotated" flies and furnishes a fourth criterion for distinguishing them. The fine lines making up these ridges form a narrow band in the normal puparia. The ridges are quite

obscure in the rotated puparia, and the lines of which they are composed form an abnormally wide band (fig. 14, 15). Also the marked elevation of the ridges which can be noted in the normal individuals is absent in the pupa cases of "rotated" flies.

When the four puparial abnormalities described above were first observed, all available material was then examined to determine the uniformity of their occurrence and their reliability as criteria for separating normal from rotated individuals prior to torsion. The pupa cases of Oregon R normal individuals were found to be identical with those of 173 known normals from *ar/ey*^{D} stock. The puparia of each of 151 rotated individuals showed all the abnormalities. No intermediates were found, and in all cases, the abnormal puparia were later seen to contain rotated pupae.

No actual rotation can be seen in the prepupae of individuals later to be rotated. Consequently, the only basis for separation of normal from rotated prepupae in the offspring of the crosses used is the character of the puparium. To test the reliability of this method, groups of prepupae from stock matings and backcrosses were examined, and those having normal puparia were separated from those with puparia showing the abnormalities described. Subsequent development was followed. Of the 116 cases examined in this manner, 50 prepupae having abnormal puparia were rotated when examined later as pupae. The 66 flies within normal puparia developed no rotation as pupae.

Prepupae

During the 11½ hour period following the formation of the puparium, the individual sheds its puparium (third instar cuticle), passes through a prepupal stage, and is transformed into the pupa (ROBERTSON 1936). The actual pupation process, marking the termination of the fourth instar (prepupal) is accomplished within the short space of 15 minutes, coincident with the release of the internal gas bubble (fig. 1, bu), which apparently assists in the separation of the pupa from the prepupal moult (fourth instar cuticle). At the end of this period, the head, thorax, and abdomen of the pupa are readily visible. Large numbers of prepupae from backcrosses expected to produce 50 percent rotated offspring were observed continuously during the quiescent period and the activities which result in their transformation into pupae. In all of the rotated cases observed, the first evidence of actual abdominal rotation appeared near the termination of pupation movements, when the pupal abdomen could first be seen clearly.

Rotated pupae were first recognized by an abnormally large space appearing between the pupa and the puparium to the right of the abdomen (fig. 2, sp). The appearance of this space and the associated twisting are the first indications of any external asymmetry in development. It will be

recalled that all the puparium abnormalities (described above) are bilaterally symmetrical in nature, and histological preparations of rotated prepupae show no torsion of either puparium or internal structures (fig. 10, 11).

Pupae

From the time of pupation, the development of the rotated flies is apparently normal, with the exception of the persistence of the abdominal torsion and the abnormal space within the puparium. BELIAJEFF'S description of the position of the pupa within the puparium holds for this material.

Histological preparations of rotated pupae fixed just after cessation of the movements of pupation show rotation of the gonads in the same direction as that of the hypodermal histoblasts (fig. 13). This torsion is also evident in later stages.

DISCUSSION

BELIAJEFF'S description of the abnormal position of the rotated pupa within the puparium has been found to hold true for the material used in the present study. The pupa assumes this twisted position at or slightly prior to the end of the prepupal period, and no rotation is evident at an earlier stage.

The striking feature of the earlier effects of this gene is its failure to produce asymmetry. The puparium abnormalities described are consistent and easily identifiable prior to rotation and are entirely symmetrical in nature. In the experimentally produced abdominal rotations described by HOWLAND (1941) torsion was evident in the larvae as well as in the puparia and adult flies. In the genetic rotation, however, we are faced with the puzzling fact that the gene influence on one stage of development brings about bilaterally symmetrical structural deviations, while only in later stages does it lead to the production of characters which swing the animal into an asymmetrical posture.

Any interpretation of these facts involves the consideration of the way in which a gene brings about the asymmetrical position of organs which, so far as can be observed, are formed in normal bilateral balance. Apparently the prime requirement in postulating a mechanism which covers this situation is the assumption that during the late prepupal stage one half of some bilateral system reacts to gene influence in a manner at variance with the behavior of the same system on the other side. So far in the course of this study no evidence has been found to indicate that any structural difference exists between the two sides of the body of the rotated individuals. Though the abdominal segments are rotated about the longitudinal axis, no anatomical unbalance has as yet been found.

It seems highly probable that the puparial abnormalities described are attributable to some specific action of the persisting larval muscles. In Calliphora, FRAENKEL and RUDALL (1940) ascribe the shortening of the larval cuticle in the formation of the puparium to muscular contraction and show experimentally that severing of muscle innervation inhibits this contraction. The abnormal bulging of the lateral puparial wall in genetically rotated prepupae seems certain to be the result of contractions of the lateral abdominal muscles.

The persistence of larval abdominal muscles in Drosophila and their relation to pupation have been described by ROBERTSON (1936). These muscles remain functional after puparium formation and are responsible for the movements of the animal during the prepupal moult (pupation). The close association in time between the movements of pupation and the development of the rotation strongly suggests involvement of the persisting larval muscles.

The first indication of differentiation of imaginal muscles in Drosophila appears in a 21-hour pupa, and the muscles are not fully formed prior to the 84th hour after pupation (ROBERTSON 1936). The specific rotational gene action is over, therefore, before the imaginal muscles appear.

Although it is indicated that the larval abdominal muscles, which persist until pupation has been completed, are in some way involved, the actual mechanism by which rotation is brought about is still undetermined. Continued investigation offers an opportunity for experimental study of the action of the larval muscles involved. Ligation of larvae to sever innervation of muscles has been suggested by the experiments of FRAENKEL and RUDALL. Further histological study of the critical prepupal period may also yield a clue to the actual forces and structures involved in the rotation of the abdomen.

SUMMARY

Puparium (third larval instar cuticle) abnormalities offering reliable means of identifying genetically rotated prepupae many hours before actual rotation occurs have been described and their symmetrical nature discussed.

Rotation has been observed to coincide with the termination of the prepupal period and the movements attendant on pupation.

Torsion of the internal abdominal organs in pupae is in the same direction and of the same degree as rotation of the external pupal segments.

Evidence suggests that the persisting larval muscles in the abdominal region are responsible for the puparial abnormalities and may also be in some way involved in the development of the rotation.

The adult muscles play no part in the development of the rotation.

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THE INHERITANCE OF TUMORS IN DROSOPHILA MELANOGASTER, WITH ESPECIAL REFERENCE TO AN ISOGENIC STRAIN OF ST SR TUMOR 36a[1]

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STUDIES of the way in which tumors are inherited in *Drosophila melanogaster* should be particularly valuable because the very extensive background of genetic knowledge and technique available for this species makes possible a more thorough analysis than could be made in any of the forms, such as mammals, usually employed in cancer research.

A number of papers appeared relatively early in Drosophila research attempting to find the location of tumor genes in various strains. The first tumor to be described was the so-called "malignant" *l(1)7* tumor (BRIDGES 1916; STARK 1918, 1919b, 1937; RUSSELL 1940). Linkage tests placed a sex-linked recessive gene, responsible for this tumor, at the locus 0.1 of the X chromosome. One hundred percent of larvae homozygous for this gene develop melanotic tumors and die before pupation. The second tumor to be described arose in this *l(1)7* stock (STARK 1919a). From this a 100 percent tumor strain was derived. A comprehensive study of its linkage relations (STARK and BRIDGES 1926) showed that 80 percent of the tumors are due to genes on the second and third chromosomes. The most effective of these is a recessive at the locus 25 on the third chromosome, and the second in importance a gene at the right end of the second near speck. The X chromosome and the fourth chromosome are responsible for 20 percent of the tumor incidence. The inheritance of a third tumor was studied by WILSON (1924). It appeared in 20 percent of larvae of an apparently homozygous strain (no change in tumor incidence in 17 generations of selection for tumors, nor in 19 generations of selection against tumors). Linkage tests indicated multiple factor inheritance, with important genes located in the right half of the second chromosome and the right end of the third. A fourth tumor was studied by WILSON (1924) which gave 100 percent tumors in a selected line homozygous for tumor genes for at least 16 generations. Linkage tests indicated at least three factors responsible, the main ones on the second and third chromosomes with modifiers on the X and fourth chromosomes.

These experiments show that many genes which influence the produc-

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tion of tumors occur in Drosophila stocks and that these may result either in 100 percent tumor incidence or less, depending upon the gene complex involved. In all, except the *l(1)7* sex-linked lethal type, multiple genetic factors contribute to the tumor formation.

In 1937 a comparison of various benign tumors and the so-called "malignant" *l(1)7* tumor was undertaken by the author. It was believed that an analysis of the differences between the benign and "malignant" tumors might demonstrate the cause of malignancy in the *l(1)7* type. A stock carrying the *l(1)7* gene was obtained and earlier data on its manner of inheritance confirmed. The other three tumors described above were no longer available, so the analysis of the manner of inheritance in benign tumors had to be started afresh on the five strains existing in 1937, none of which had been analyzed.

TUMOR STOCKS USED IN THESE EXPERIMENTS

The available benign tumor stocks were (1) *st sr es ro ca tu 36a*, referred to here as *st sr;* (2) *bw tu;* (3) *aa tu 36e;* (4) *lz³/f;* and (5) *gt bb¹/ClB*. In each

TABLE 1

STOCK	NUMBER LARVAE EXAMINED	NUMBER WITH TUMORS	% TUMOR LARVAE
st sr	903	445	49
bw tu	606	189	31
aa tu 36e	198	56	28
lz³/f	671	102	15
gt bb¹/ClB	263	18	7

strain a low percentage of the flies develop melanotic tumors during the latter part of larval life (third and fourth days, 25°C). The tumors are all very similar in structure and growth and do not hamper development of the animal, since they stop growing abruptly at pupation (RUSSELL 1940). The incidence of tumors observed in each stock is given in table 1.

ELIMINATION OF GENETIC HETEROGENEITY AS CAUSE OF PHENOTYPIC VARIABILITY IN TUMOR PRODUCTION

All of these five tumor stocks are like WILSON'S 20 percent tumor strain in that only a certain percentage of the flies develop tumors. Is this due to genetic heterogeneity in the stocks or to non-genetic causes? In WILSON'S work selection for and against tumors did not change the incidence of tumors in 17–19 generations, so it is quite certain that the variability was due to non-genetic causes. In the present five stocks a certain amount of inbreeding had been practiced in maintaining the stocks, but it was not close, and no selection for tumors had been made. Two sets of preliminary

tests of four of the stocks and a conclusive test of the fifth are given here.

Single pair crosses of tumor×tumor and non-tumor×non-tumor flies were made in the *bw tu* and *lz*^{3}/*f* stocks to see if there was any difference in the tumor incidence in their progeny. The adults were examined for tumors within one day after emergence, but some difficulty in finding tumors was experienced owing to the pigmented body of the adult. The results of this experiment are given in table 2.

In neither case is the proportion higher when the parents are tumorous; in fact, the tendency is in the other direction.

In two other tumor stocks, *aa tu 36e* and *gt bb*^{1}/*ClB*, an attempt was made to increase the tumor percentage by selection, which would be effective only if there was heterozygosity of tumor genes. To avoid the diffi-

TABLE 2

STOCK	INCIDENCE IN OFFSPRING OF NON-*tu*×NON-*tu* CROSS			INCIDENCE IN OFFSPRING OF TU×TU CROSS		
	NO. ADULTS EXAMINED	NO. WITH TUMORS	% TUMOR ADULTS	NO. ADULTS EXAMINED	NO. WITH TUMORS	% TUMOR ADULTS
bw tu	272	93	34	104	28	27
lz^3/*f*	324	35	10	83	3	4

culties encountered earlier in observing tumors through the adult's pigmented chitin, the observations were all made in the larval stage. Eggs were collected from four cultures of the *aa tu 36e* and from seven cultures of the *gt bb*^{1}/*ClB* stock. Larvae hatching from these eggs were raised in translucent strained-banana-agar cultures and examined daily for tumor masses. Only cultures which produced tumors were subcultured; the secondary cultures were tested by the same method, and those producing tumors were again subcultured. This experiment was discontinued after four generations, because selection seemed to have no noticeable effect in increasing tumor incidence, since in the fourth generation in each stock the incidence of tumors was equal to or lower than the incidence in the original unselected stock. Rather than attempting further to increase tumor incidence in these four low tumor stocks it was decided it would be more profitable to concentrate on an analysis of the highest tumor stock *st sr*.

In neither of the two sets of experiments just described is there any evidence of genetic heterogeneity as the cause of the phenotypic variability in production of tumors, but these studies are far from constituting definite negative proof. The best way to test whether or not heterozygosity was responsible would be to get a completely homozygous tumor strain and

study the incidence there. In the experiments mentioned in the introduction, as in most genetic experimentation, homozygosity could be achieved only by many generations of inbreeding. Fortunately homozygosity can now be produced in Drosophila in three generations (four to six weeks) by crossing a desired type to a stock with a balanced lethal pair and a dominant marker pair on each chromosome (MULLER 1936). The *ClB/+; Cy/Pm; D/Dfd* stock, controlling all the chromosomes except the minute fourth, was obtained from DR. P. T. IVES, and females from it were crossed to a tumor-bearing male from the high-tumor *st sr* stock. By this method, a homozygous (except for the fourth chromosome) strain, carrying the tumor genes present in the original *st sr* father, was developed. Eggs were collected from this pure strain and the development of 647 larvae observed. Of these, 266, or 41 percent developed tumors. The percentage in the non-fixed general *st sr* stock is shown in table 1 to be of the same order of magnitude, 49 percent. The failure to produce 100 percent tumors in the homozygous strain carrying all of the genes of the original tumor-bearing male proves that non-genetic factors influence the production of tumors in this strain, unless there is a continued heterozygosity in the minute fourth chromosome of factors causing tumors, a very unlikely contingency.

ANALYSIS OF THE TUMOR-PRODUCING EFFECT OF THE X, SECOND, AND THIRD CHROMOSOMES OF THE ST SR STRAIN

The genetic factors influencing tumor formation have been studied further in the *st sr* strain. This analysis differs considerably from those of BRIDGES and WILSON. They looked for tumors in adult flies resulting from outcrosses to stocks whose chromosomes bore marker genes. A tendency for tumors to be scarce in combination with one of those markers indicated the presence in the tumor stock of a tumor gene near the locus of that marker. This method has the advantage of localizing the tumor genes within general regions of the length of the chromosomes. It has two disadvantages: (1) large numbers carrying any particular combination of chromosomes from the tumor and marker stocks cannot be obtained, preventing accurate counts of tumor incidence in these special combinations; and (2) tumor counts cannot be made in the larval stage, since identification of other characters must be made in adults.

The method used in this study was an extension of the MULLER balanced-lethal-marker method. The effect of each of the three major chromosomes of the *st sr* stock on tumor incidence was determined by producing from the tumor-bearing *st sr* stock and the tumor-free marker stock, stocks fixed for the desired chromosome combinations. This made possible the observation of large numbers of animals and counts of tumors in the larval stage, both of which should tend to make these results more accurate than

those from earlier studies. However, in its present form, this method does not give any indication of the localization of tumor genes within any chromosome, so it falls short of the former method in this particular.

The stocks listed in column 1 of table 3 were obtained by this MULLER method (x^t, 2^t and 3^t refer to the X, second, and third chromosomes, respectively, of the homozygous *st sr* stock). There are four different stocks containing 2^t, three containing x^t, and four containing 3^t. The tumor incidences in each stock are given in the second, third, and fourth columns of table 3.

Apparently the second chromosome of this tumor stock must be homozygous in order for any tumors to appear. The average incidence in all stocks containing $2^t/2^t$ is 48 ± 1 percent. In all without $2^t/2^t$ it is zero. This indicates on the second chromosome of this tumor stock the presence of a major gene or genes necessary for the production of tumors.

TABLE 3

GENOTYPE	NO. LARVAE EXAMINED	NO. WITH TUMORS	% TUMOR LARVAE	S.E.	DIFF. FROM TOTAL $2^t/2^t$	S.E. DIFF.	DIFFERENCE/S.E.
x^t/x^t; $2^t/2^t$; $3^t/3^t$	647	266	41	±3	− 7	±3	2.33
x^t/x^t; *Cy/Pm*; $3^t/3^t$	72	0	0				
x^t/x^t; $2^t/2^t$; *D/Dfd*	512	328	64	±2	+16	±3	5.33
ClB/+; $2^t/2^t$; $3^t/3^t$	281	87	31	±3	−17	±3	5.66
ClB/+; $2^t/2^t$; *D/Dfd*	460	239	51	±3	+ 3	±3	1.00
ClB/+; *Cy/Pm* = $3^t/3^t$	92	0	0				
All with $2^t/2^t$	1900	920	48	±1			

However, the other chromosomes also influence the tumor incidence, provided the second chromosome tumor genes are present to make tumor production possible. The last three columns of table 3 show that the percentage of tumors in each individual $2^t/2^t$ stock, except *ClB*/+; $2^t/2^t$; *D/Dfd*, is significantly different from the grand total percentage in all $2^t/2^t$ stocks combined (difference over standard error of difference more than two).

What is the effect of each of the other two chromosomes studied? The effect of the X chromosome may be found by comparing all of the $2^t/2^t$ stocks having x^t/x^t with the corresponding ones having *ClB*/+. In each case the x^t/x^t stock gave a higher tumor incidence than its *ClB*/+ partner. Combining all those with x^t/x^t, the incidence is 51 ± 1.5 percent (594/1159). In those without x^t/x^t the incidence is 44 ± 1.8 percent (326/741). The difference between these ratios is 7 percent, its standard error 2.3 percent, so the difference is significant. This indicates that the X chromosome of the

st sr tumor stock contains a gene or genes which have a significant effect in increasing the number of tumors developed in $2^t/2^t$ stocks.

The effect of the third chromosome may be found similarly by comparing all the $2^t/2^t$ stocks having $3^t/3^t$ with those having *D/Dfd*. In each combination table 3 shows a lower percentage in the $3^t/3^t$ stock than in its *D/Dfd* partner. The incidence in all such $3^t/3^t$ stocks combined is 38 ± 1.6 percent (353/928), while in those without $3^t/3^t$ it is 58 ± 2.3 percent (567/972). The difference between these two values is 20 ± 2.3 percent and is significant, since it is 8.9 times its standard error. Thus the third chromosome of the *st sr* tumor stock contains a gene or genes which have a significant effect in decreasing the number of tumors developing in $2^t/2^t$ stocks.

To summarize the information on the inheritance of tumors in the benign tumor stock, *st sr es ro ca tu 36a*, we now know that there is on the second chromosome a recessive gene or genes essential to the production of any tumors, on the X chromosome a gene or genes increasing tumor incidence, and on the third chromosome a gene or genes decreasing tumor incidence.

DISCUSSION

This experiment differs from earlier work on the inheritance of tumors in Drosophila in two ways: (1) The stocks were built up to study each chromosome of the tumor stock separately. (2) The counts were all made in the larval stage in an attempt to increase accuracy. The chief result was proof that both non-genetic and multiple genetic factors influence the production of tumors in the *st sr* tumor stock.

At the present time very little indication has been found as to the types of external or internal environmental factors exerting the non-genetic influence, but this should be a fertile field for further investigation. One factor noted by earlier workers was scarcity of food or some other crowding condition, since crowded cultures often show few or no tumors. Crowding cannot be the only environmental factor influencing tumor production, however, since in our experiments the tumors were counted in larvae growing with a liberal food supply in uncrowded conditions, 10–25 larvae in a 50 mm Petri dish, and the incidence of tumors was nevertheless 41 percent rather than 100 percent. Temperature at which larvae grow also might influence tumor incidence and would be worth testing in a laboratory equipped for temperature experiments. The larvae counted in these experiments grew in an incubator at $25 \pm 0.5°C$, indicating that variation in temperature is not necessary to produce variation in appearance of tumors. The recent work of HARTUNG (1942) indicates that X-radiation may act as an environmental factor increasing tumor incidence.

The analysis of genetic factors influencing tumor incidence in this homozygous stock indicates that different regions of this particular gene

complex differ greatly in their effects on tumor incidence, the second chromosome being essential (against this genetic background, at least) to the production of any tumors, the X increasing the incidence, the third decreasing the incidence. Theoretically it should be possible to extend the MULLER method to localize these effects within shorter regions of the chromosomes and eventually, by crossing-over experiments, to locate the genes involved. This seems difficult, since tumor counts are much more accurate on the larval stage, and few genes have a recognizable effect on the larval phenotype. A technique could be developed for separating tumor and non-tumor larvae among offspring of linkage crosses, and determining crossover percentages after these emerge as adults. This seems to the author a profitable field for future work.

It would be profitable, also, to apply this type of analysis to other relatively high tumor stocks such as *bw tu*, to see what sort of genetic determination is involved there. Eventually it might be possible to combine the tumor-influencing genes or regions of the chromosomes of two such stocks, and study the effects of their genes in combination.

CONCLUSIONS

Since a completely homozygous strain developed from a tumor-bearing male gave only 41 percent tumors, it is clear that non-genetic as well as genetic factors influence tumor incidence in the *st sr es ro ca tu 36a* stock.

There is on the second chromosome of this *st sr* stock a recessive gene or genes essential to the production of any tumors in this stock.

The net effect of the X chromosome of the *st sr* stock is to increase the incidence of tumors, provided the second chromosome tumor genes are present.

The net effect of the third chromosome of the *st sr* stock is to decrease tumor incidence, provided the second chromosome genes are present.

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DIPLOID-TETRAPLOID PERICLINAL CHIMERAS AS BUD VARIANTS IN CITRUS[1]*

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INTRODUCTION

NUMEROUS genetic variations affecting portions of Citrus trees have occurred under cultivation. "Bud-variation" or "bud-mutation" forms have been studied extensively from the horticultural viewpoint in various commercial varieties, such as the Eureka and Lisbon lemons (SHAMEL et al. 1936), but little is known about the causation of the changes concerned.

Certain conspicuously mixed bud-variation forms, such as lemon "strains" with white-margined leaves (SHAMEL 1932) and orange strains with two kinds of fruit rind (SHAMEL et al. 1925), appear to be essentially periclinal chimeras which include the parent type as one component. In other cases, as with the horticulturally important Wase satsuma (TANAKA 1932), it seems very doubtful whether frequent somatic reversion of a bud-variant form to the parent type is due primarily to an originally chimeral condition or to genetic instability of the new type.

The extreme diversity of Citrus forms and the very abundant heterozygosis which seems to be general in this genus (WHITE 1914, FROST 1926) suggest that gene mutation occurs comparatively often. This process may therefore be considered as one probable cause of bud variation in Citrus, although the conditions are unfavorable for its discrimination from somatic crossing over or structural aberrations in chromosomes.

"Twin" variations in rind characters, which change adjacent sectors in opposite directions from the parental condition, are occasionally observed. These presumably indicate the occurrence of abnormal mitosis. When, as occasionally happens, both rind thickness and rind color are changed in opposite directions in two adjacent sectors, some shift involving more than one gene locus seems clearly indicated. Aside from such cases (and germ-layer shifts in chimeras, of course), the general nature of bud variation in Citrus seems likely to be determinable only when a cytologically visible alteration is involved.

Of the possible kinds of cytological evidence on the causation of bud

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variations in Citrus, evidently only changes in number of chromosomes are likely to be detected at all readily. Microsporocytes and root tips of stem cuttings presumably show the chromosome number only of two different germ layers from which they are derived. Their combined evidence, with bud-variation forms, therefore needs to be supplemented, especially by direct or indirect determination of the number of chromosomes in the epidermis derived from the first germ layer. These detailed investigations are necessary in order to distinguish between entirely modified variants and chimeras.

In recent years many investigations have been carried out to determine the number of germ layers in the primordia of plants. SCHMIDT (1924) proposed the "tunica-corpus" theory, visualizing two main germ regions, and FOSTER (1936, 1939) has shown that the number of germ layers participating in the development of the various tissues varies considerably in different species. BLAKESLEE et al. (1939) and SATINA et al. (1940, 1941), working with various colchicine-induced chimeras in Datura, have clearly demonstrated that three germ layers take part in the development of this plant. Apparently nothing is known about this subject with regard to Citrus.

Few determinations of chromosome number are available for Citrus forms of known bud-variation origin. NAKAMURA (1929) examined microsporocytes of two variant branches of satsuma classed as Wase. He found the same chromosome number ($2n = 18$) in these branches as in the "normal" part of the same trees and observed no difference in chromosome behavior. The Shamouti orange of Palestine, which probably originated from the Belladi orange by bud variation and which frequently produces branches of Belladi (OPPENHEIM 1929), has been found to have the normal number of chromosomes in microsporocytes (OPPENHEIM and FRANKEL 1929).

Under at least some climatic conditions tetraploidy is common among Citrus seedlings produced asexually (through nucellar embryony) by diploid parents of many varieties (FROST 1925, 1938; LAPIN 1937). Since triploids, but no tetraploids, have been found among seedlings of known gametic origin (F_1 interspecific hybrids) in the same cultures with nucellar tetraploids, it seems that the doubling of chromosome number which produces tetraploidy in Citrus seedlings must usually occur prior to the beginning of the embryonic divisions. Frequent cases of production of both tetraploid and diploid seedlings by the same seed strongly suggest that the doubling usually occurs in the pistil, and probably in the nucellus.

Tetraploid branches on otherwise diploid Citrus trees, indicating the probable origin of tetraploidy in shoot meristems, do not seem to have been reported prior to the present study. On the basis of the tree charac-

teristics of the bud-variation forms which have been studied in California, it is probable that tetraploid bud variations are rare in Citrus. In the case here reported, a small portion of a seedling tree was partially tetraploid, and the change in chromosome number probably occurred after the germination of the seed.

MATERIAL AND METHODS

From a hybrid mandarin produced by pollination of King mandarin by Dancy tangerine at the UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION, 128 seedlings were grown from 111 seeds resulting from open pollination. On examination in January, 1932, at the age of about four and one-half years, all these plants seemed identical with the seed parent as to genetic type, and therefore all presumably had originated asexually by nucellar embryony. On one of these trees, however, one small branch (later designated "b"), which arose from the trunk at the height of about one and one-half meters, bore four lateral shoots, of which the largest was observed to have broad, apparently thick leaves, suggestive of tetraploidy. No description was recorded for the other three laterals. After the new shoots and leaves of the following spring had developed, branch b was examined again. Among a total of 13 shoots bearing two or more leaves each, only one then seemed to be diploid, ten appearing tetraploid, and two possibly intermediate. The entire variant branch died after the tree was transplanted.

The remainder of the seedling tree (designated "a") seemed to be entirely diploid. The progeny trees a-1 to a-3 of the following discussion were propagated from two shoots distant from branch b. Two pairs of trees (b-1 and b-2, b-3 and b-4) were propagated from two nonadjacent shoots on branch b. Trees a-1 to b-4 were all budded on trifoliate-orange rootstocks and were grown in consecutive order in the nursery. Tree b-5 was budded on sweet-orange rootstock from one of the bud sticks mentioned. In 1936 these trees were set in the orchard, in the order a-1 to b-5.

In August, 1940, leafy cuttings were taken from scattered terminal shoots of four trees, a-1, a-2, b-1, and b-4, and placed in sand in a closed hotbed with electric bottom heat. Rooted cuttings were potted in October and kept in a greenhouse.

In January, 1941, root tips from some of these cuttings were fixed in the Craf fixing solution (RANDOLPH 1935) for 24 hours, dehydrated by the tertiary butyl alcohol method (JOHANSEN 1940), and embedded in paraffin; sections 6 microns thick were stained with Heidenhain's haematoxylin. All drawings were made with camera lucida at table level at a magnification of approximately 3900×.

In April of the same year flower buds of different sizes were collected

from trees a-1, b-1, and b-4 (a-2 had no buds, probably because of overproduction of fruits in the preceding season) and fixed for 12 hours in three parts alcohol:one part acetic acid and then stored in 70 percent alcohol; smears were made with acetocarmine (concentrated solution in 45 percent acetic acid) and heated nearly to boiling before sealing the cover glass. Chromosome counts were made in dividing pollen mother cells with approximately the same magnification as used for counts in root tips.

At the same period the tips of fast growing shoots and small central portions of mature leaves were also fixed in Craf, dehydrated as were the

TABLE 1

Thickness of leaves on trees from which cuttings were taken.

TREE	LEAF THICKNESS (MM×100)		
	MEAN	DIFFERENCE	DIFF./S.E.
a-1	27.25±.26		
a-2	27.70±.44		
b-1	36.32±.34	8.62	15.4*
b-4	37.45±.31	9.75	18.1†

* Difference between a-2 and b-1 highly significant (t=2.71 for n=40 at P=0.01. GOULDEN, 1939, table 94).

† Difference between a-2 and b-4 also highly significant.

Difference between b-1 and b-4 hardly significant—Diff./S.E.=$\frac{1.13}{0.46}$=2.46 (t=2.42 for n=40 at P=0.02).

root tips, and cross-sectioned at 8 to 10 microns; they were also stained with Heidenhain's haematoxylin.

MORPHOLOGY OF BUDDED PROGENIES

General characters of trees and leaves; fruiting habit

In February, 1941, trees a-1 to a-3 had a considerable crop of fruit (very heavy on two trees, light on one) and appeared to be typical specimens of the seed-parent form in all respects (fig. 1). Of the five trees budded from branch b, trees b-1 and b-5, especially the former, were relatively short and broad, and they had not more than four fruits each (suggesting the relative unfruitfulness characteristic of tetraploids); b-1, at least, had foliage which seemed clearly indicative of tetraploidy and was especially broadened, as a result of very low branching (fig. 2). Trees b-2 to b-4, representing both bud sticks from branch b, were tall and erect, about like trees a-1 to a-3 in shape, but on the whole were intermediate

in yield of fruit (b-2 and b-3 about medium, b-4 very light) (fig. 3). However, much if not all of their foliage seemed to indicate tetraploidy, as did that of b-5.

FIGURE 1.—Progeny tree a-1. Erect and typical of seed-parent form.

FIGURE 2.—Progeny tree b-1. Foliage is indicative of tetraploidy. Broadening of tree shape is probably exaggerated by very low branching after budding.

FIGURE 3.—Progeny tree b-4. Similar in shape to a-1 but with most of the foliage indicative of tetraploidy.

Leaf thickness

Twenty mature leaves, similarly located and of similar size, were collected in April, 1941, from each of the trees from which cuttings had been taken. Two measurements of thickness were made near the middle of each leaf, with a coverglass gauge reading in hundredths of a millimeter. The means and their standard errors are given in table 1.

The means for trees b-1 and b-4 are compared with that for tree a-2, which has higher mean and variability than a-1, for determination of the significance of the differences. The leaves from both of the trees propa-

TABLE 2

Relative breadth of leaves of cuttings.

TREE	NUMBER OF LEAVES	LEAF-SHAPE INDEX		
		MEAN	DIFFERENCE	DIFF./S.E.
a-1 and a-2	23	2.035±.025		
b-1	19	1.916±.030	0.119	3.05*
b-4	12	1.880±.032	0.155	3.78†

* Difference between general average of (a-1 and a-2) and b-1 is significant ($t=2.71$ for $n=40$ at $P=0.01$).

† Difference between the same average and b-4 is also significant ($t=2.72$ for $n=35$ at $P=0.01$).

Difference between b-1 and b-4 is not significant: Diff./S.E. $=0.78$ ($t=2.76$ for $n=29$ at $P=0.01$).

gated from the variant branch (b) were significantly thicker than those of the trees propagated from other parts (a) of the parent seedling. On the other hand, the difference between the two "b" trees was hardly significant, and that between the two "a" trees was not significant.

Leaf index

To secure an unbiased sample for determination of the shape index (length/width) of the leaf blade, all available original leaves (omitting seriously distorted ones) on the cuttings were measured. The means and standard errors are given in table 2.

Results for 50-leaf samples of other varieties, previously studied at the Citrus Experiment Station, indicate that the differences here found, in spite of their statistical significance, are not highly significant in relation to the actual chances of sampling from replicate trees. The differences, however, indicate greater relative width of leaves on the trees budded from the variant branch. Eleven tetraploid Citrus forms grown at the Citrus Experiment Station all showed greater leaf breadth than the corresponding diploids, the differences usually being much greater than those here presented.

CYTOLOGY AND HISTOLOGY

The study of the above-mentioned morphological characters of the progeny trees of the variant branch b suggests very strongly that it originated through chromosome doubling. To test this hypothesis, chromosome counts were made in root tips of cuttings, at meiosis, and later in young leaves and stems; the size of stomata was determined; and the general histology of leaf primordia, mature leaves, and young shoots was studied.

Chromosome number in root tips of cuttings

As mentioned above, chromosome counts were made in root tips of cuttings which were taken from trees a-1, a-2, b-1, and b-4. Special atten-

TABLE 3

TREE	NUMBER OF CUTTINGS EXAMINED	NUMBER OF ROOTS EXAMINED	CHROMOSOME NUMBERS
a-1	1	3	$2n=18$
a-2	1	3	$2n=18$
b-1	4	15	$4n=36$
b-4	4	21	$2n=18$

tion was paid to secure counts in several cell layers of the meristematic tissue. With the exception of two cases, the same number was found in all

layers of a particular root tip. In one of the exceptions, apparently two diploid nuclei, one of which appeared as a metaphase polar view with approximately 18 chromosomes, were dividing in one single cell surrounded by tetraploid ($4n=36$)[4] tissue. In another instance one single tetraploid cell was found in otherwise entirely diploid tissue.

Excluding the above exceptions the following results were obtained:

As expected, trees a-1 and a-2 produced normal diploid roots on their cuttings (fig. 4); b-1 originated tetraploid roots (fig. 5), and b-4, rather unexpectedly, had diploid roots on its cuttings.

Chromosome number at meiosis

In the spring of 1941, trees a-1, b-1, and b-4 produced a small number of flower buds, which were examined for their chromosome numbers at meiosis. The counts were made at MI, AI, and especially at MII and AII. Furthermore, observations were made on the presence of laggards, the number of microspores derived from each microsporocyte, and the variability of pollen size. The results were as follows:

Tree a-1: No chromosome counts could be made, but the number of microspores derived from each microsporocyte was, with rare exceptions, four, and the pollen was noted to be normal and uniform in size.

Tree b-1: The exact number of chromosomes at the different phases of the division of the microsporocytes could be determined only in a limited number of cases, since clear distinctions were often not possible between univalent, bivalent, and multivalent chromosomes. At MI usually from 14 to 18 apparent bivalents could be observed, including possible multivalents in instances of less than 18; at AI various sets (two in each cell) of 16 to 17 apparent univalents were counted; at this stage and at TI occasional laggards were encountered; in one MII configuration two plates of approximately 14 univalents were counted, several laggards being distributed in the cytoplasm; at various second anaphases groups of 17 and 18 chromosomes were observed; at two of these phases, however, groups of only nine chromosomes were found. Only 70 percent of 200 examined microsporocytes gave rise to four apparently normal microspores, 0.5 percent giving three, 21.5 percent five, and 8 percent six microspores. Mature pollen grains were variable in size.

Tree b-4: Various counts showed nine apparent bivalents at MI, two groups of nine suspected univalents at AI, and nine univalents at MII phase and AII. At two first metaphases, however, approximately 18 apparent bivalents were counted. Of the examined microsporocytes 87 percent developed into four microspores, 13 percent into five; the pollen

[4] In this paper "n" (here=x) is used exclusively to designate one genome, not to distinguish between reduced (n) and somatic (2n) conditions.

grains were noted to be uniform in size, perhaps a little more variable than the ones from tree a-1.

These results indicate that tree a-1 has normal diploid microsporocytes and b-1 apparently tetraploid ones. The data concerning tree b-4 are too scanty for a definite conclusion regarding the cytological constitution of its microsporocytes. However, since tetraploids vary with respect to their chromosome behavior at meiosis (FROST 1938), and since it is not always possible to make definite distinctions between uni- and multi-valent chromosomes at meiosis of Citrus, tree b-4 may also have tetraploid microsporocytes. The data above mentioned which disagree with this assumption may be due to errors regarding the number of chromosomes intimately associated at meiosis, or to a shift in germ-layer composition within tree b-1 or b-4.

Size of stomata

Stomata measurements were made under low magnification by means of a micrometer ocular; for that purpose freehand sections of lower epidermis were prepared, two from each side of the midrib in the central portion of each of ten leaves collected at random on the south side of the three budded progeny trees, and fixed in formalin-acetic-alcohol (5–5–90; 50 percent alcohol). The sections were examined without staining; from each leaf 20 stomata (about five from each section) were measured, totaling 200 stomata for each progeny tree. The area of each stoma was calculated by multiplying the product length×width of the area occupied by the two guard cells, by $\pi/4$, assuming that this area has the shape of an ellipse. The variance "between" and "within" leaves was calculated for each tree, and BRIEGER'S (1937) table of ζ (giving the probabilities of ratios of standard deviations) was used for evaluation of the results. This study indicated that the variances mentioned were not significantly different. Consequently, the total variability in stomatal area for each tree is used in estimating the standard error of that tree's mean stomatal area. The mean areas and their standard errors are presented in table 4.

In spite of the fact that the above statistical analysis reveals significant differences in mean stomatal area between trees a-1 and b-1 and also between trees b-1 and b-4, it is concluded that these differences must be due to some environmental factor and not to chromosome doubling, since they did not agree with actual chromosome counts in the epidermis of leaf primordia, as presented later, which revealed that this cell layer is for all three trees diploid; they also disagreed with observations of the width of the epidermal cells of mature leaves, which was found to be equal for all three trees. Unpublished investigations under way at the INSTITUTO AGRONÔMICO revealed recently that the differences of mean stomata areas in diploid and tetraploid epidermis of otherwise genetically identical Citrus

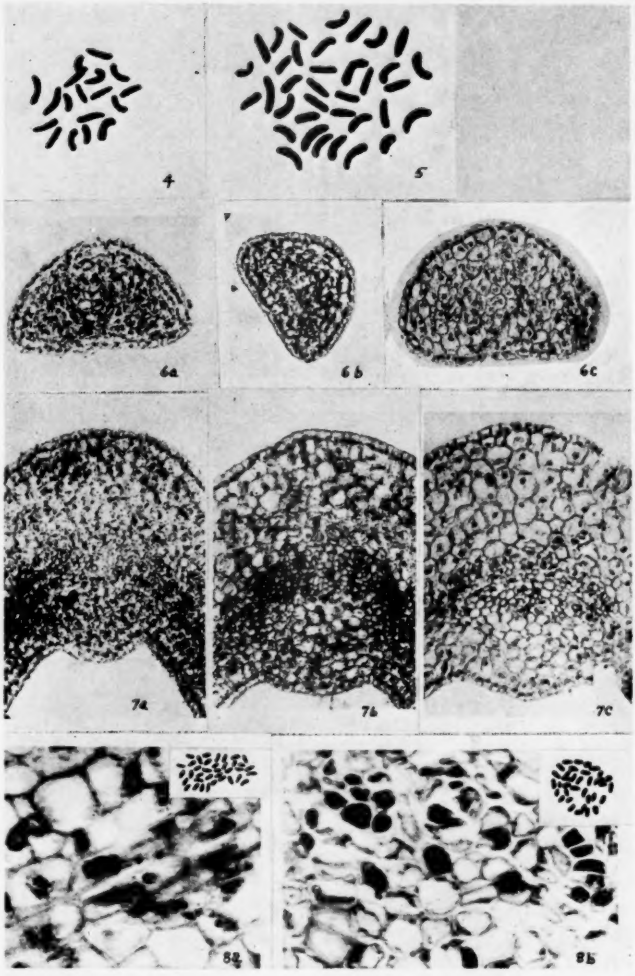

FIGURE 4.—Metaphase chromosomes in root tip of cutting from progeny tree a-1 (2n=18). ×2600.

FIGURE 5.—Metaphase chromosomes in root tip of cutting from progeny tree b-1 (4n=36). ×2600.

FIGURE 6.—Cross sections of leaf primordia (×133): a—from progeny tree a-1; b—from progeny tree b-1; c—from progeny tree b-4.

FIGURE 7.—Cross sections through midrib region of young leaves (×133): a—from progeny tree a-1; b—from progeny tree b-1; c—from progeny tree b-4.

FIGURE 8.—Procambium cells in young leaves of progeny tree b-4 (×667): a—4n=36 in procambium of leaf blade; b—4n=36 in procambium of midrib region.

forms are about four times as large as those mentioned for trees a-1 and b-1 in table 4.

Leaf primordia

The sectioned material permitted the study of the histology and the determination of the chromosome number in different cell layers of very young leaf primordia and in later stages when the leaf blade was already partially developed. Very young leaf primordia appear in cross sections as disk-like structures of a few more or less concentric cell layers. A little later two protuberances, one on each side, begin to project outward, forming the blade of the leaf. At this stage the vascular tissue of the mid-

TABLE 4

*Mean area of stomata in micrometer units.**

TREE	NUMBER OF STOMATA	AREA IN SQUARE UNITS		
		MEAN	DIFFERENCE	DIFF./S.E.
a-1	200	94.42±1.09		
b-1	200	106.38±0.97	11.96	8.19†
b-4	200	93.76±0.87	0.66	0.47‡

* The actual area of stomata in square microns is obtained by multiplying these means by 2.89, as each unit of the micrometer ocular corresponded to 1.7 micron.

† Difference between means of a-1 and b-1 is highly significant.

‡ Difference between means of a-1 and b-4 is not significant.

Difference between means of b-1 and b-4 is also highly significant (Diff./S.E. = 9.71) (t = 2.60 for n = 200 at P = 0.01).

rib also starts to develop, and in some cross sections procambial regions are easily detected in the blade, which are connected with the vascular tissue of the midrib. The differentiation into spongy and palisade parenchyma occurs much later.

The following differences were found among the sectioned material of the three trees:

A-1 (*Fig. 6a and 7a*). In round-shaped cross sections the cells of the epidermis are approximately of the same size as the internal cells. In later stages, due to the fact that the epidermis develops into a more organized layer, its cells usually become a little longer in the radial direction, appearing somewhat smaller than the others. The cells of the parenchyma tissue on the dorsal or abaxial side of the midrib become much larger than the epidermal cells. The cells of the procambium in cross sections of the blade are narrow and elongated, containing densely stained cytoplasm. In material of a-1, as expected, 2n = 18 was determined in most cell layers.

B-1 (*Fig. 6b and 7b*). The development of the leaf primordia is, of

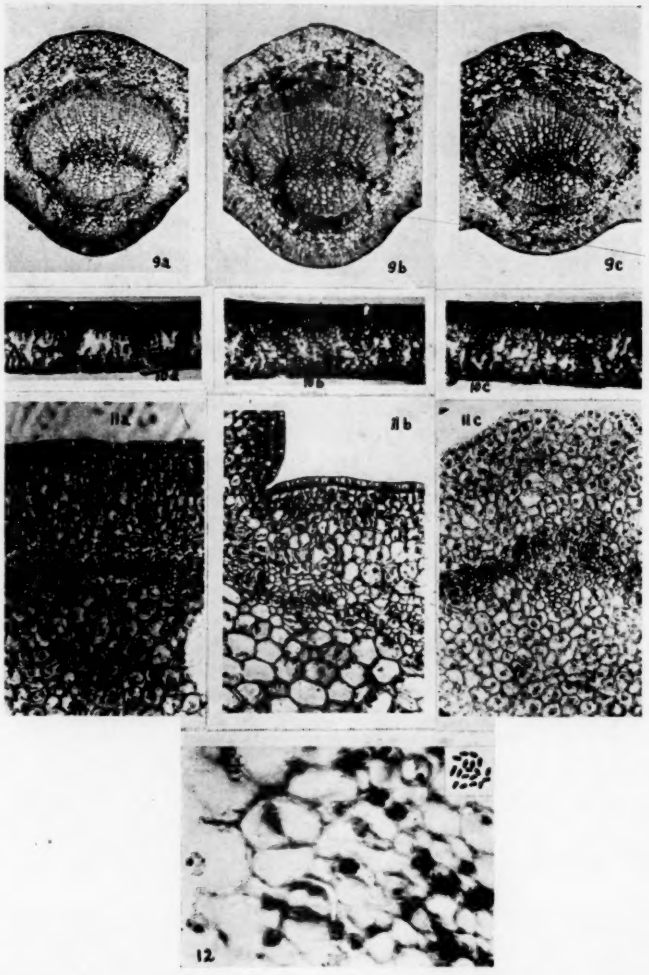

FIGURE 9.—Cross sections of midrib regions of mature leaves (×37): a—from progeny tree a-1; b—from progeny tree b-1; c—from progeny tree b-4.

FIGURE 10.—Cross sections of blades of mature leaves (×37): a—from progeny tree a-1; b—from progeny tree b-1; c—from progeny tree b-4.

FIGURE 11.—Cross sections of portions of young stems (×133): a—of progeny tree a-1; b—of progeny tree b-1; c—of progeny tree b-4.

FIGURE 12.—Portion of the pith and procambium of a young stem of progeny tree b-4; 2n = 18. ×1427.

course, identical with that in a-1; the differences in cell size, however, are as follows: the subepidermis and adjacent layers are formed by cells which, from the beginning, are much larger than the epidermal cells; the difference is further evident upon comparison of the epidermis and the adjacent parenchymatous tissue of the dorsal side of the midrib of somewhat older leaves; the size difference is here more conspicuous than in tree a-1. The procambium cells, on the contrary, do not seem to differ in size from those observed in tree a-1. Extensive search to determine the chromosome number in the various layers of these cross sections revealed that the epidermis is diploid ($2n = 18$), the subepidermis and adjacent layers tetraploid ($4n = 36$), and the procambium cells also tetraploid ($4n = 36$).

B-4 (*Fig. 6c, 7c, and 8a, b*). No appreciable differences in cell size and structure could be detected between leaf primordia of tree b-4 and those of tree b-1; the chromosomal constitution of the cell layers was the same as for b-1—that is, the epidermis was diploid ($2n = 18$), while the subepidermis, the adjacent layers, and the procambium were tetraploid ($4n = 36$).

Mature leaves (*fig. 9a, b, c and 10a, b, c*)

The study of cross sections through the central portion of mature leaves, including the midrib and parts of the blade, furnished the following information: the epidermis is formed by cells of approximately the same size in all three plants, a-1, b-1, and b-4; the palisade parenchyma is definitely wider in b-1 and b-4 than in a-1, the cells being, in average, longer and broader in the former two; the spongy parenchyma occupies more space and its cells are larger in b-1 and b-4 than in a-1. At the midrib region the palisade and spongy parenchyma are much more developed in b-1 and b-4 than in a-1; in the vascular tissue the vessels, tracheids, etc., apparently have a larger diameter in b-1 and b-4 than in a-1. No appreciable differences in size of cells could be detected between the material of b-1 and that of b-4.

Young stems (*fig. 11a, b, c and 12*)

Cross sections of the terminal portions of young shoots were examined. The cells of their epidermis were found to be of similar size in all three plants, a-1, b-1, and b-4; the cortex is made of larger cells in b-1 and b-4 than in a-1. In these young shoots no differences in cell size could be detected in the procambium and vascular tissue which begins to be formed at this region. In material examined from trees a-1 and b-1 a considerable difference in size was found between the cells of the pith and of the cortex, the former having cells about twice as large as the latter; in tree b-4, however, this difference in cell size of these two regions was insignificant, all the cells being, on the average, of the same size. The following chromosome counts were made: the epidermis, as expected, was diploid for all

three trees; the parenchymatous cells of the cortex were tetraploid for b-1 and b-4, diploid only for a-1; the procambium and pith cells of trees a-1 and b-4 were diploid, these tissues being tetraploid only for b-1.

DISCUSSION AND CONCLUSIONS

The results of the above investigations indicate that the original variant branch, found on one of the progeny trees of a hybrid mandarin (King ×Dancy), owed its peculiar characters to the occurrence of somatic chromosome duplication and has produced buds of at least two types of cytological constitution. Both are periclinal chimeras, and assuming that, as in Datura (BLAKESLEE et al. 1939, 1941; SATINA et al. 1940, 1941), three germ layers participate in the development of the vegetative shoot of Citrus, these chimeras are of the following nature: one is $2n-4n-4n$, respectively, for its first, second, and third germ layers, and gave origin to progeny tree b-1; the other, originating progeny tree b-4, is $2n-4n-2n$ for the same layers. It cannot be determined when the duplication occurred, whether in a bud of the original parent tree or further back during its ontogeny, nor can it be determined how the differentiation into these two chimeras took place.

Based on the cytological constitution of these two chimeras, the following considerations may be presented:

The first germ layer takes part only in the formation of the epidermis; differences in size of stomata between two individuals, in spite of being of statistical significance, are not always an indication of differences in chromosome number of the epidermis. Caution should be exercised therefore in making deductions regarding the chromosomal constitution of this layer, based on the size of its stomata.

The second germ layer is responsible for the formation of the internal leaf tissue, including the procambium and the vascular tissue, as was clearly demonstrated by chromosome counts in the procambium of leaf primordia for plant b-4, whose second layer, and only this one, is tetraploid.

The similarity of leaf thickness and of leaf shape, as indicated by the leaf-shape index of trees b-1 and b-4 (tables 1 and 2) constitutes further proof that the cytological constitution of the leaves of these trees is identical. This fact is not in accordance with the findings of SATINA and BLAKESLEE (1941), since in Datura the vascular tissue of the leaves is derived from the third layer.

The fact that the leaves of the chimeras are indistinguishable and that they resemble very closely the leaves of true tetraploid plants, in spite of having a 2n epidermis, is an indication of the outstanding role which the second germ layer plays in the formation of Citrus leaves.

The cell layers underneath the epidermis in the young stem (cortex)

are also derived from the second layer. As in other plants, this layer is responsible for the formation of the microsporocytes. Since it is tetraploid in both chimeras, it produces in them the relative unfruitfulness characteristic of tetraploid Citrus forms.

All tissues derived from the procambium and later the cambium of the stem and its pith originate from the third germ layer. The fact that this layer is diploid in one of the chimeras (b-4) and tetraploid in the other (b-1) probably explains why these two progeny trees differ in growth habit, b-4 being tall and erect and about like a-1 in shape, and b-1 short and broad. If this explanation is valid, it may be concluded that the third germ layer plays an important role in the determination of the growth habit of the trees. Chromosome counts in root tips of cuttings of the two chimeras have proven that these roots, which develop from a callus, are always derived from the third layer. It has been observed that callus at the base of a stem cutting forms beneath the bark and appears to arise from the fascicular cambium alone.

The breadth of the leaves was found to be smaller for the two chimeras than usually in 11 Citrus forms, presumably tetraploid throughout, which have been studied at the CITRUS EXPERIMENT STATION; this difference may be due to the diploid nature of the epidermis of the chimeras.

In progeny tree b-4, as stated above, the vascular tissue of the leaves is tetraploid, the corresponding tissue in the stem, however, being diploid. Since, according to several investigators, the procambial cells in leaf primordia differentiate in two directions—namely, upward toward the leaf apex and downward toward the shoot apex—to form the vertical leaf traces, it may be concluded that in tree b-4 the tetraploid leaf vessels probably unite with the diploid stem vessels at the insertion region of the petiole.

Since both chimeras owe their origin to somatic chromosome doubling which occurred in nature and since they cannot easily be distinguished by their morphological characters from true tetraploids, it is obvious that great care must be taken in selecting tetraploid Citrus seedlings for study or breeding. The evidence, however, indicates that partially tetraploid trees are probably rare.

Further germ-layer studies on Citrus are needed, particularly with reference to the generality of the two-layer pattern of leaf ontogeny which we have found in one form, and with reference to the germ-layer relations of those morphologically complex structures, the pistil and the fruit. It is not evident how the two-layer pattern could prevail in the leaves of such forms as SHAMEL'S (1932) variegated lemon, since these leaves are mainly white-margined, with variegated interior, and therefore evidently must have two genetic types within the epidermis (FROST in press).

SUMMARY

The present investigation deals with the cytological nature of a bud variant which originated on a progeny tree of a hybrid mandarin (King ×Dancy). From the variant branch several progeny trees were grown in comparison with others derived from the normal part of the tree. The results of extensive investigation indicate that the budded progeny of the variant branch includes at least two types of chimeral constitution: one is 2n–4n–4n respectively for its first, second, and third germ layers, and the other is 2n–4n–2n for the same layers.

On the assumption that three germ layers exist in Citrus, it was demonstrated that the first germ layer forms the epidermis, the second one all leaf tissues (with the exception of the epidermis), the microsporocytes and at least part of the cortex of young vegetative shoots, and the third one forms the procambium, cambium, and the pith of the stem. Determination of chromosome number in root tips of cuttings of these two chimeras, demonstrated that these roots, at least when they derive from a callus, have their origin in the third germ layer. Comparison of stomata of mature leaves of different plants indicates that size differences, even if they are of statistical significance, are not always due to differences in chromosome number in the epidermis. The progeny trees of these chimeras differ considerably in growth habit, the one with a diploid third germ layer being approximately normal (erect), the other with a tetraploid third layer being low and broad. This fact suggests that the third layer plays a considerable role in determining the growth habit of the trees. Both chimeras are rather unfruitful, which is typical for Citrus tetraploids.

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BIVALENT PAIRING IN AN INDUCED TETRAPLOID OF TRADESCANTIA

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THE tetraploid forms of any genus are usually separated into two groups, auto- and allopolyploid, depending on the behavior of the chromosomes at meiosis. The meiotic behavior is also used as an indication of phylogenetic history. Those forms that produce bivalents at meiosis I usually are assumed to be the result of interspecific hybridization followed by chromosome doubling, while those forms that exhibit multiple configurations at meiosis I are assumed to be intraspecific hybrids followed by chromosome doubling. DARLINGTON (1937) proposes an alternative to this by postulating that an autotetraploid may show bivalent pairing if enough time has elapsed to allow the accumulation of internal rearrangements so that there no longer remains the necessary attraction between the four homologues to result in quadrivalent formation. The induced tetraploids in Tradescantia show that other factors—namely, the time of chromosome doubling and the degree of structural heterozygosity in the parents—may also influence the behavior of the chromosomes of the induced tetraploid at the first meiotic division.

An attempt was made to induce polyploidy in the genus Tradescantia by means of thermal shock, following the methods of RANDOLPH (1932). The equipment used for this work has been described by SAX (1937). The experiment was designed to produce a controlled polyploid and to compare this with the known parents. The parent plants were two diploid clonal lines of natural hybrids between *Tradescantia canaliculata* and *T. humilis* originally collected by DR. EDGAR ANDERSON at Austin, Texas, and propagated under his accession numbers of Oak Hill 5 and Oak Hill 13. These plants are completely self-sterile and set no open-pollinated seed under greenhouse conditions. When these plants are hand cross-pollinated, each capsule contains an average of 3.5 seed.

SAX found that sudden changes in temperature were as effective as extremes of temperature for the production of polyploid tissue; thus the parent plants were preconditioned at 39–40°F for a few hours immediately following inter-pollination and then were quickly shifted to the temperature of 95–97°F for varying lengths of time. The treatments were repeated daily until the first pollinated flowers had begun to mature the hybrid seed. The time elapsing between pollination and the administering of the thermal shock was varied considerably, in an attempt to induce chromosome doubling at the first division of the hybrid zygote. The ar-

rangement of the flower clusters is such that the treatments given developing zygotes would also effect developing gametes at the two meiotic divisions. At maturity the seeds were collected and bulked. Germination was poor, and less than fifty percent of the seeds produced plants. The plants were grown to maturity under field conditions. The chromosome number of each plant was determined from the haploid microspore division using the acetocarmine technic. Many of the plants died or did not bloom, but among the 338 plants which flowered 311 were diploids, 18 were triploids, six were tetraploids, and three were aneuploids with either 17 or 19 chromosomes.

Oak Hill 5 and 13 are normal diploids with good cytological behavior. Each of the parents possesses a single centric fragment, which appears to be inherited through the female side and which is slightly deleterious to the rate of development of the pollen tube. There were very few segregates containing more than one fragment. The parents also possess a series of genes for chlorophyll deficiency, which appear to be complementary rather than allelic. There were various degrees of chlorophyll deficiency, varying from true lethal albinos to barely perceptible virescence. Associated with the chlorophyll deficiency is a less vigorous growth rate. There was no association between the chlorophyll deficiency and the presence or absence of fragments which would indicate that the chlorophyll deficient genes were borne on the major chromosomes. Occasionally unbalanced gametes functioned as indicated by the aneuploid types produced. The parents also possessed a number of genes effecting sterility, because the sterility of the progeny varied from 5 to 100 percent, and most of it could not be accounted for by chromosomal irregularities.

A random sample of the diploid segregates was examined for meiotic behavior. Chromosome pairing was normal, with only occasional univalents and inversion bridges which are characteristic for most species of Tradescantia. The triploid segregates showed the typical trivalent, bivalent, and univalent distribution found in spontaneous triploids. Five of the six tretraploid plants were similar to normal tetraploid species in their meiotic behavior. From 50 to 60 percent of the chromosomes were paired as quadrivalents, with the remaining chromosomes forming trivalents, bivalents, and univalents (*cf.* ANDERSON and SAX 1934).

One of the tetraploids was distinctly different from the other tetraploid segregates. In this plant, designated as #91, most of the chromosomes were paired as bivalents. Only 5.6 percent of the chromosomes were associated as quadrivalents, and the occasional univalent—2.5 percent—were distributed regularly, suggesting precocious terminalization of pre-existing chiasmata. The number of chiasmata per chromosome was 0.9. The frequency of inversion bridges was 3.7 percent. Pollen sterility was relatively

low—8.4 percent. A comparison of the parents and the tetraploid is shown in table 1.

The cytological behavior of the induced tetraploids indicates two types of origin. Since most Tradescantias are heterozygous in respect to chromosome structure (SWANSON 1940), the production of a tetraploid by meiotic irregularities resulting in diploid gametes would produce a plant in which no two of the four genomes are identical. The lack of preferential pairing would favor quadrivalent formation. In plant 91, however, the meiotic behavior suggests that chromosome doubling occurred at the time of fertilization or during embryonic development. Somatic doubling in a plant known to be heterozygous in chromosome structure would result in two sets of duplicated genomes. At meiosis there would be preferential pairing of duplicated genomes, and only occasionally would chromosomes of unlike genomes pair to form quadrivalents or pair as bivalents and produce inversion bridges. Most of the bivalents would consist of identical chromosomes.

TABLE 1

Comparison of induced tetraploid #91 with its diploid parents.

	OAK HILL 5	OAK HILL 13	#91
Diameter of PMC at metaphase I	29.5μ	30.8μ	44.6μ
Width of mature pollen	33.7μ	34.0μ	43.3μ
Resting nucleus measured in long axis of root cell	17.4μ	15.3μ	25.2μ
Length of stomata guard cell	48.8μ	56.4μ	70.3μ
# stomata/mm² of leaf	92.3	110.3	60.6
# xta. per cell	10.7	10.8	21.4
# xta. per chromosome	0.89	0.90	0.89
% inversion bridges at anaphase I	5.8%	6.5%	3.7%
% pollen sterility (abnormal)	21.0%	17.0%	8.4%

Since the temperature shock was given at various times during floral development, the induced polyploids could be derived from chromosome doubling in the gametes or from chromosome duplication in the fertilized egg or during embryonic development. The production of diploid gametes could result either from premeiotic polyploidy or from meiotic irregularities. The triploid segregates must have been derived from diploid gametes produced by one of the parental plants. The origin of the tetraploid segregates is indicated by their meiotic behavior.

The interpretation of bivalent formation in an autopolyploid is supported by the evidence from other sources. Thus in the diploid form of *Primula floribunda*×*P. verticellata* the chromosomes pair as bivalents, but in the somatically derived tetraploid the chromosomes still pair as bivalents. This pairing is primarily between chromosomes of similar

genomes with only a slight tendency for cross pairing or the formation of quadrivalents (DARLINGTON 1937). Preferential pairing also may occur within a species. In the triploid Drosophila and Rumex (DARLINGTON 1937) and in the tetraploid Melandrium (WARMKE and BLAKESLEE 1940) the X chromosomes show preferential pairing, although the X and the Y chromosomes pair regularly in the normal diploid forms. Preferential pairing also occurs when the opposing chromosomes come from the same species as has been demonstrated by many workers. Chromosomes tend to pair more frequently with their duplicated identical partners than with the partially homologous partners with which they previously paired. In the presence of identical chromosomes, the latent attraction between the semihomologous chromosomes is neglected and only bivalents are formed as a rule. Among the species with a higher chromosome number there remains, however, a minor tendency for the residual attraction to be expressed, and this results in the formation of an occasional multivalent (*cf.* DARLINGTON on polyploid species of an intermediate type, and KATTERMAN (1931) on *Bromus erectus*).

In one species, *Allium Schoenoprasum*, LEVAN (1935) has shown that two distinct cytological types occur. One is a giant autotetraploid by definition and the other is a smaller allotetraploid. This has been interpreted by DARLINGTON as an example of the change of an allotetraploid into an autotetraploid by the progressive elimination of all of those characters by which one parent of the allotetraploid was distinguished from the other parent; or perhaps the converse has occurred and the species arose as an autotetraploid with free pairing among the homologues resulting in quadrivalents. The latter condition still remains in some of the forms. The others have changed in several ways and have lost their gigantism as well as the autopolyploid pairing due to cytological differentiation arising between the different pairs of the haploid sets. A method by which this latter type of change could come about without any genetic change that is physiologically expressed is by inversions coming into the chromosomes.

If the history of Plant 91 were not known definitely, there would be a tendency for one to classify this type of polyploid as an allotetraploid, because chromosome pairing is predominantly in the form of bivalents. In a somatically double plant there is a certain amount of cross pairing detween non-duplicated chromosomes and a low percentage of quadrivalent associations. The amount of cross pairing is a funetion of the frequency and size of the interstitial inversions in the original diploid form. Crossing over in the non-duplicated chromosomes and their subsequent segregation as chromatids would result in the formation of non-identical gametes. Irregular disjunction of the quadrivalents formed, while not seen in #91, might possibly lead to an upset of the condition necessary for bivalent formation

in subsequent generations. Species exhibiting this type of behavior would closely approximate DARLINGTON'S definition of polyploid species of an intermediate type (*cf.* DARLINGTON 1937, table 30).

The duplicated type of chromosome behavior demonstrated by #91 is not found among the wild populations of Tradescantia. It cannot exist except by asexual propagation in a self-sterile species. In a natural colony the chances of two plants of an approximately similar cytological makeup simultaneously doubling their chromosomes in the somatic tissue to result in the formation of bivalents and then intercrossing is extremely remote. If crosses between a somatically doubled and a gametically doubled tetraploid form occurred, the resulting progeny, while tetraploid, would not have the necessary identical chromosomes to give the type of pairing found in #91. If only one plant were produced in a colony of diploids, the resulting progeny would be triploids. In either case the precise condition required for the type of chromosome association found in #91 would be lost irretrievably. This limitation may be removed in certain species where the induced tetraploid condition restores self-fertility (STOUT and CHANDLER 1941). Allosyndesis in an autopolyploid also could occur in a structurally heterozygous species in which the plants are self-fertile although normally cross-pollinated.

The origin of an autopolyploid Tredescantia which behaves as an allopolyploid cytologically may explain in part the heretofore anomalous cytological behavior of those species which possess polyploid forms but show no evidence of polyspecific ancestry (MUNTZING 1936).

SUMMARY

Two types of tetraploids were derived from a diploid plant by temperature changes during gametic and embryonic development. Five of the tetraploids behaved as autopolyploids with somewhat more than half of the chromosomes pairing as quadrivalents. These presumably were derived from the union of diploid gametes. One of the induced tetraploids behaved as an allopolyploid with chromosomes pairing primarily as bivalents. The origin of this plant is attributed to chromosome doubling following fertilization. The difference in chromosome behavior is attributed to structural heterozygosity in the parental genomes.

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THE DEVELOPMENT OF DOMINANT RUMPLESSNESS IN CHICK EMBRYOS

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STUDY of a genetic variant usually concerns three fairly distinct problems. The first is, necessarily, that of defining its nature—its morphological or physiological effect upon the organism. The second deals with the mechanism by which it is transmitted from one individual to others. The third problem involves the study of the development of the variant in order to determine when, where, and how the normal ontogeny is modified to produce the final effect.

The first two problems for a mutation which causes a rumpless condition in chickens have been analyzed (DUNN 1925; LANDAUER 1928; DUNN and LANDAUER 1925, 1934, 1936). The mutation results in a loss of caudal vertebrae and attendant tail structures and is transmitted as a simple dominant (*Rp*). The question as to when and how the tail is reduced or lost has as yet received no unequivocal answer. LIBON (1911) and DU TOIT (1913) studied the development of rumplessness in chicks and arrived at opposing conclusions. The former claimed that there is a degeneration of the normally formed tail during the ninth and subsequent days of embryonic life. The latter, on the other hand, showed fairly clearly that the tails of the chicks which he studied were reduced in size as early as the fourth day of development. Unfortunately his material did not extend to stages sufficiently early to give information concerning the cause of this reduction.

The present report is an attempt to supply an answer to the remaining problem for the mutation analyzed by DUNN and LANDAUER—namely, when is the tail lost or reduced in the Rumpless chicks and what is the mechanism by which it is lost?

MATERIAL AND METHODS

Eggs for this work were obtained from two matings of the dominant Rumpless stock at the STORRS EXPERIMENT STATION. One of these was a mating of an heterozygous male by heterozygous females ($Rp+ \times Rp+$); the other was a mating of a normal Leghorn male by heterozygous females ($++ \times Rp+$). The latter mating was used because of the low fertility resulting when two *Rp* animals mate. Most of the eggs were laid in trapnests so that their pedigrees were known. Eggs from normal Leghorn matings were used for comparison. Optimum incubation conditions were obtained through the use of a large forced draft incubator.

The eggs were opened at desired stages (hours of incubation stated in the text are for total time in the incubator), and the embryos were fixed in either Bouin's or Kleinenberg's solutions. They were then stained with borax-carmine and cleared in cedar-wood oil. Each embryo was examined after it had been cleared; somites were counted, the caudal end observed, and an outline sketch was made by tracing around a projection of the animal. After this, the embryos were embedded and sectioned at 7, 10, 12, or 15μ depending on the stage. Transverse and sagittal sections were used. Some were then restained with alum-haematoxylin and counterstained with orange-G.

Since the *Rp* stock used was one in which modifiers towards the normal were present (resulting in intermediate rumpless conditions, DUNN and LANDAUER, 1934, 1936), a number of animals were allowed to develop to hatching. The rumps were stained with alizarin red S and cleared by a modified Spalteholtz technique in order that the degree of rumplessness of the skeleton might be studied and the range of variation for this condition be available for comparison with the embryo material.

OBSERVATIONS—GROSS

Observations were made on 378 embryos from the *Rp* matings and 84 from the normal Leghorn stock. These confirm DU TOIT's description for embryos of four days and older. Embryos which will give rise to Rumpless chicks may be recognized by the absence of a tail. The one significant contribution that we can make in this regard arises from the action of the genetic modifiers mentioned above. Although these were presumably present in DU TOIT's animals, he probably had insufficient cases to demonstrate them; one of his 12 rumpless embryos does show what seems to be an intermediate condition.

At the end of the fourth day of incubation, tail development ranges from nearly normal to complete absence. A continuous series of reduction has been found. The tail may vary from a slightly shorter and blunter than normal appendage to a small rounded mass a short distance posterior to the hind limbs (fig. 2). As DU TOIT pointed out, there is a reduction in the number of somites: normal embryos at this age (and later) may have from 47 to 52 or more somites. DU TOIT's rumpless embryos (four to eleven days) had from 33 to 36 somites. Among our embryos (fourth and fifth days) which could be classified by gross examination as showing some degree of rumplessness the range was from 32 to 43 somites. Some of the higher grade intermediates may have escaped detection.

The only other grossly visible evidence of abnormal development was the presence of a pouching or vesiculation at the end of the nerve cord. This could be seen in both extreme and intermediate cases.

The relation between the anterior axial structures and the appendages was normal even in the extreme cases. Thus, somite #19 was opposite the fore-limb bud and somite #31 was opposite the hind-limb bud. There was no distortion of the axial pattern other than that found in the tail.

Table 1 gives the data for the distribution of the different classes of embryos (four and five days) for the two crosses. These data include those found for the various classes among newly hatched chicks. The same parents are involved in the two groups. The absence of extreme *Rp* cases

TABLE 1

CROSS	NORMAL	INTERMEDIATE*	EXTREME
Embryos (4 & 5 day)			
$++\times Rp+$	17	22	10
$Rp+\times Rp+$	10	13	8
Chicks			
$++\times Rp+$	26	31	0
$Rp+\times Rp+$	12	16	6

* All grades

in the newly hatched chicks of the $++\times Rp+$ mating probably is due to poor sampling. The number of cases involved is too small to justify any conclusions concerning segregation ratios, but when the total *Rp* population (regardless of grade) is compared with the normals, the expected ratios are roughly obtained.

The tail of three days embryos is not very long; growth in length is just beginning. Therefore no distinction between *Rp* and normal embryos, based on size difference of the tails, can be made. In some embryos which had been incubated for 70 hours or more the distal region of the nerve cord was distended and considerably vesiculated; similar to the four day embryos in which the pouching was associated with tail reduction. The pouching was sufficiently marked to be clearly seen in transparent and, in a few cases, in opaque specimens. It was not until the embryos were sectioned that this feature could be linked with others to indicate the first grossly visible evidence of the onset of the *Rp* condition. Pouching of the nerve cord was seen in younger embryos, even normal ones. It was not until the end of the third day, however, that, in embryos destined to be *Rp*, the pouching became so pronounced as to be definitely unlike any of the normals.

DU TOIT has classified one 72 hour embryo as rumpless. His conclusion was based on the number of somites and the fact that the embryo looked retarded. It was not sectioned. This one embryo had 34 somites and was compared to ten embryos (70–73 hrs.) described by KEIBEL which had from 34 to 42 somites (average 38). In our material no difference in somite num-

ber between *Rp* and normal embryos could be detected at the end of the third day of incubation. In 31 embryos from the Rumpless matings, which had been incubated for 70–74 hours, the range was from 28–38 somites, with 21 of them having either 33, 34, or 35 somites. In 24 normal Leghorn embryos, incubated for the same length of time, the range was from 27–37 somites, with ten embryos having 33, 34, or 35 somites. If anything, this indicates a slightly greater number of somites for the *Rp* stock. This is probably due to sampling. A clearer indication that the somite reduction has not begun by the end of the third day is seen from the analysis of sections. The embryos of this age whose somite number is small do not necessarily have the other features which are considered diagnostic for the *Rp* condition, whereas some of those with the greatest somite counts may have these features.

Embryos of two and two and one-half days of incubation could not be distinguished from the normal by gross examination.

OBSERVATIONS—SECTIONS

In his micro-anatomical studies Du Toit found that his rumpless embryos varied from the normal in four features. The most obvious was a reduction in somite number. In addition the nerve cord was very irregular and pouched at its termination, the notochord was very thick and bent ventrally at the distal end, and both ended abruptly without merging with an indifferent mass of cells; the indifferent mass was not found at all.

Table 2

AGE	*Rp* MATINGS	LEGHORNS
5 days	15	2
100–105 hours	15	0
90– 99 hours	15	3
80– 89 hours	0	0
70– 79 hours	37	23
60– 69 hours	11	0
50– 59 hours	37	11
40– 49 hours	7	1
30– 39 hours	20	4
Total	157	44

Embryos from our collection were sectioned to see whether or not they showed the same structural abnormalities as Du Toit's and to extend the observations in order to discover the reasons for the abnormalities. For this study 157 embryos from the Rumpless and 44 from the Leghorn collection were selected. Table 2 shows the age groups into which these embryos were distributed. When the embryos were sufficiently far along so that the degree of rumplessness could be ascertained grossly, nearly equal

numbers of the three conditions (normal, intermediate, and extreme) were sectioned. The earlier embryos had to be taken at random.

Fourth and fifth days

Since the tail structure of embryos of the fourth and fifth days of incubation proved to be very similar, they will be treated together.

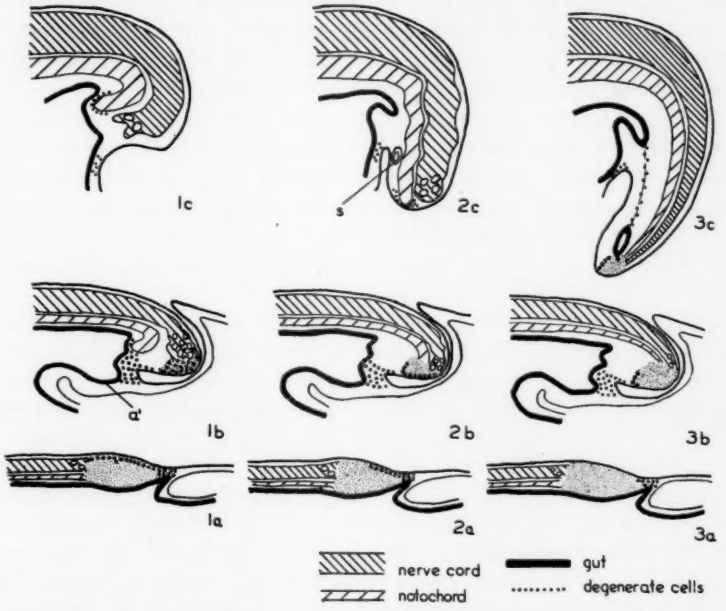

FIGURE 1.—Diagrammatic sagittal sections illustrating the important morphological events in the development of Rumpless embryos. 1, extreme rumplessness; 2, intermediate rumplessness; 3, normal; a, latter part of second day of incubation; b, latter part of third day; c, latter part of fourth day; s, abortive somite; a', allantoic diverticulum. Fine stippling indicates the indifferent tail mass. Small circles in distal portion of the nerve cords represent multiple canals.

Nerve cord: In both the intermediate and extreme Rumpless embryos the nerve cord ended in a distended tube which frequently had more than one canal for some distance. There were many side pouches, and the walls were irregular. This condition was more pronounced in the intermediates than in the extreme Rumpless embryos. In addition, the cord, instead of being straight, was wavy for some distance before it terminated (fig. 1–2c). The termination was abrupt, without any merging with the indifferent mass of so-called mesoderm. Normally the nerve cord tapers gradually and smoothly and runs, without definite boundary, into a small mass of indifferent cells which contributes to all of the tail structures (fig. 1–3c).

(This description does not differ from that of Du Toit.) In our material the distortion of the nerve cord was usually much less marked in the extreme *Rp* embryos, where the distal neural tissue frequently appeared eroded, than in the intermediates. In two of the more normal intermediates some of the indifferent tail tissue was present and was full of necrotic cells (these will be discussed below). In one case the embryo looked normal superficially, but the nerve cord was irregular.

Notochord: As in Du Toit's material, it was found that the notochord of Rumpless embryos was very thick and blunt at the distal end and stopped abruptly without merging with the indifferent mass. Normally the notochord tapers gradually and loses itself in this mass (fig. 1–3c). In our material, too, the notochord bent ventrad sharply a short distance before terminating (fig. 1–1c). In four of our extreme cases this bending was so marked that the notochord actually pushed into the cloacal portion of the gut (fig. 6); the gut epithelium was pushed before it. Invariably the distal tip of the notochord was surrounded by pycnotic and some degenerate cells.

Somites: The somites of the tail were quite normal, except for a decrease in number, until near the region of nerve cord distortion. Here, frequently, were several small vesicles, undoubtedly abortive somites, which were either above, below, or to one side of the nerve cord and notochord (fig. 1–2c).

Gut: Normally, there is a posterior extension of the gut in these stages. This post-anal or tail gut has a lumen for a short distance posterior to the cloaca, then dwindles to a thin line of degenerate cells until near the tip of the tail where a canal with a lumen is found again (fig. 1–3c). The post-anal gut was missing from the extreme Rumpless embryos, and only the filament of degenerate cells could be found in the low grade intermediates.

Degenerating cells: There have been many descriptions in the embryological literature of cells containing chromophilic granules of various sorts. These have usually been interpreted as cells which were in process of degeneration. Since cells of this type play an important part in the genesis of the Rumpless condition, we shall digress at this point to describe those found in our material. Chromophilic granules or globules (fig. 4) are invariably found in the cytoplasm of such cells. Occasionally there are homogeneous globules which do not take chromatin stains; these, presumably, are precursor stages to the later ones. The globules may either be completely stained with the chromatin stain, or a varying amount of the globule may be clear while the rest takes the chromatin stain. Frequently these globules have a signet-ring appearance (not unlike the signet-ring chondriosomes found in some insects). The chromatin staining portions react positively to Feulgen stain. There may be only one globule in a cell

or many. Some greatly enlarged cells with as many as a dozen granules have been seen. These definitely resemble macrophages, but may occur in stages where macrophages supposedly do not. Nuclei are present in these cells; they may appear normal in cells with one or two globules or shriveled in those with many. When globules occur only in occasional cells (the usual situation) only one is present in each cell. Less frequently there are large foci of degenerating cells. In such foci it is often difficult to see individual cells (except at the periphery); all that can be seen is a large mass of chromophilic granules (fig. 3). Either the cells are so closely packed that cell boundaries cannot be distinguished or the cells have broken down and extruded the granules.

While a thorough search for degenerating cells in regions anterior to the tail was not made in the present material, many were observed in both normal Leghorn embryos and embryos from the Rumpless matings. In four and five day embryos from both sources such isolated cells were observed in the nerve cord (more found in posterior regions, but many seen anteriorly), brain, notochord, somites, ganglia, dermatome, scleratome, and mesenchyme. Light concentrations of degenerate cells were frequently encountered in the dorsal part of the distal nerve cord, in the Wolffian ducts at the point where they enter the cloaca, in the hind-gut, in the mesoderm around the base of the allantois, in the central portion of the post-anal gut, in the hind-limb buds (in several cases they were seen in the center of the mesoderm, in one in the distal mesoderm and ectoderm), and in the indifferent cell mass of the tail tip. This latter focus is not present in either the intermediate or extreme Rumpless embryos since the indifferent mass is not present. In these, scattered degenerate cells, together with true pycnotic cells, were found around the distal tip of the blunt notochord. As mentioned previously, in the two intermediates in which some of the indifferent mass remained, the mass was full of degenerate cells. In several intermediates the distal part of the nerve cord (the distorted region) had concentrations of these cells.

Third day. 68–76 hours

Nerve cord: Of the forty-eight embryos from the *Rp* matings in this group nine had undistorted nerve cords. Twenty-two had some degree of abnormality at the distal end; usually this consisted of a distention of the neurocoel followed by a region where there were two or three canals with little or slight bulging and pocketing of the surrounding neural tissue. In 17 the nerve cord was considerably distorted at the distal end. From five to eight canals were present, and the tissue around these canals was very thick and irregular. The nerve cord frequently was irregular and distended anterior to the multiple canals. At this stage even the distorted

nerve cords merged with the cells of the indifferent mass. As may be seen in table 3, a certain amount of distortion occurs normally in the distal region of the nerve cord. Of 23 normal Leghorn embryos in this age group, 13 had multiple canals. None were so markedly irregular as the extreme cases found in the embryos from the *Rp* matings.

Notochord: The notochord in embryos from the *Rp* matings in most cases did not differ from the normal condition. At this stage the notochord normally becomes somewhat thicker where it merges with the indifferent mass. In several of the Rumpless embryos the notochord was even thicker than normal and ended abruptly, without merging with the indifferent mass. The notochords of these embryos (fig. 1–1b) bent sharply and came

TABLE 3

CONDITION OF NERVE CORD	NO. OF CASES	CONCENTRATION OF DEG. CELLS IN N.C.			CONCENTRATION OF DEG. CELLS IN INDIFF. TAIL MASS		
		NONE	MILD	EXTREME	NONE	MILD	EXTREME
Rp matings							
No distortion	9	9	0	0	4	5	0
Slight distortion	22	17	3	2	6	14	2
Extreme distortion	17	1	4	12	0	2	15
Leghorn							
No distortion	10	8	2	0	4	6	0
Slight distortion	13	10	1	0	9	2	0
Extreme distortion	0	0	0	0	0	0	0

into contact with the bulge which separates the hind-gut from the allantois; they are undoubtedly fore-runners of such cases as the four embryos, described in the previous age group, in which the notochords pushed into the cloacae.

Somites: Abnormalities of the somites were not observed at this age. Apparently somite formation was still going on or had just stopped, since some of the embryos had as many somites as were found in later extreme *Rp* embryos and none had so many as the less extreme intermediates and normals. The range of somite numbers, as previously indicated, was almost identical with that for normal Leghorns of the same age group.

Gut: There was no post-anal gut in embryos of this age. The hind-gut was a blind sac which was in contact with the mesenchyme of the proximal portion of the tail-bud.

Degenerating cells: As in older embryos, cells with chromophilic granules were frequently encountered in various parts of the body in both normal embryos and those from our *Rp* matings. A few scattered cells of this type were seen in brain, anterior nerve cord, somites, scleratome, dermatome,

mesenchyme of various parts of the body, liver diverticula, the distal ends of the Wolffian ducts, the distal end of the notochord, and, very consistently, in the sero-amnionic raphe. Large concentrations of degenerating cells were encountered only in the tail region, and these are of marked interest for the present study.

The allantoic diverticulum at this stage is a shallow trough of endodermal epithelium which is directed somewhat downward and anteriorly. Posteriorly the gut epithelium is continuous with that of the hind-gut at the region where the sub-caudal pocket pushes forward and forms an anteriorly directed bulge of all tissues. At this point the mesenchyme surrounding the allantois and hind-gut makes contact with the mesenchyme of the proximal portion of the tail-bud. Here, too, the gut is closest to the ectoderm and forms the so-called cloacal membrane or anal plate; and here the ectoderm of the amnion is continuous with that of the tail-bud. The tissues, especially the mesoderm, of this cloacal membrane region were full of degenerating cells. All of the mesoderm around the base of the allantois, at the cloacal membrane, and ventral to the hind-gut had many cells which were full of chromophilic globules. These cells extended into the mesoderm of the proximo-ventral portion of the tail-bud. Many were found in the endodermal cells of the hind-gut and the base of the allantois; and many were found in the ectoderm at the mid-line of the region where the amnionic ectoderm joins the body ectoderm. The lumen of the hind-gut was frequently full of degenerating cells. The above description applies to all embryos, both from the *Rp* matings and the Leghorn stock (fig. 1–1, 2, 3 b). There was variation in the degree of concentration of such cells in both stocks, but more embryos showing extreme concentrations were found in the group from the *Rp* matings.

Another focus of degenerating cells was found in the indifferent mass of the tail-bud (table 3). There was a marked variation in the number of such cells in this mass—from an occasional cell with only one globule (fig. 1–3b), to a mild concentration along the ventral periphery of the mass (fig. 1–2b), to a condition in which virtually the entire indifferent mass was full of large degenerating cells (fig. 1–1b; 3). The former two conditions were encountered in eight of 23 Leghorn embryos. In most of these the cells were restricted to the proximal region of the tail-bud and were continuous with the focus of degenerating cells around the cloacal membrane. Where these cells were actually in the indifferent mass they were few and scattered near the periphery. The extreme condition was found only among embryos from *Rp* matings, and of these 15 of 17 were associated with extreme distortion of the nerve cord. In 12 of these 17 embryos the distal portion of the nerve cord itself (fig. 5) and its lumen were full of degenerating cells. In several such cases there was a region where the ectoderm in the mid-

ventral line near the base of the tail was actually eroded away, and degenerate cells seemed to be extruding from this spot (fig. 7). Bending and thickening of the notochord was observed only among these extreme cases. The least extreme concentrations were found in embryos in which the nerve cord was undistorted.

After this description was written, the author was informed of a discussion of the role of degenerating cells in cloaca formation in Gallinaceous birds by BOYDEN (1922). The latter's description is almost identical with the present. Two foci of phagocytic cells (BOYDEN identifies them as macrophages) are described: one at the region of the cloacal membrane, associated with the formation of the fenestrations which precede the formation of the bursa of Fabricius; the other at the peripheral region of the indifferent tail tissue. In reference to the latter I quote (page 168): "The proximal half, on the other hand, is degenerating. Some of it may contribute to the mesenchyme of the tail, but most of it, as indicated by the presence of innumerable phagocytes gorged with pycnotic nuclei, is undergoing resorption." The present work differs only in relation to the number of degenerating cells found in the indifferent mass of normal embryos, but this is undoubtedly due to a difference in relative quantities since the author's figure 1–3b is almost identical with BOYDEN'S (1936) figure 5.

51–59 hours

Nerve cord: Multiple canals were encountered at the distal tips of the nerve cords in nearly all embryos of this age from both groups. All 11 normal Leghorns had them, as did 35 of the 37 embryos from the *Rp* matings. This condition varied, in both stocks, from two to five or six small canals. There was no additional distortion of the nerve cord; the region of multiple canals was just slightly thicker than more anterior regions of the cord.

Notochord: There was no difference in the notochords of the two stocks. Two or three chromophilic granules were seen at the distal tip of nearly every notochord.

Somites: Somites were normal in both groups. Their number varied from 20 to 28 in the *Rp* group and from 20 to 29 in the normal Leghorn embryos.

Gut: During this age interval the tail-fold is either not present or, in the oldest embryos, is just beginning to form. As a consequence the tail anlage is represented by a thickening in the posterior region; this tapers rather sharply to a point where all layers are in intimate contact at the mid-line (presumably the remnant of the primitive streak). The area of contact extends for some 80 to 100μ posteriorly and then merges with the extra-embryonic tissues. The gut is open until the region of fusion is reached and here forms a postero-dorsally directed pouch (figs. 1–1a, b, c), the

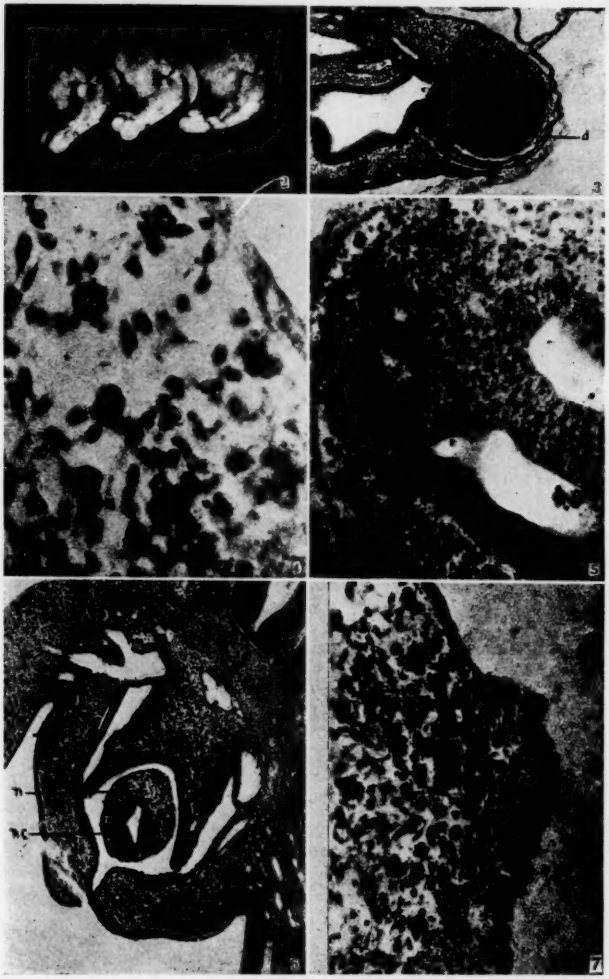

FIGURE 2.—92½ hr. embryos showing normal, intermediate and extreme rumpless tails.

FIGURE 3.—71½ hr. *Rp* embryo. Sagittal section (oblique) showing extreme concentration of degenerating cells in the indifferent tail mass at d.

FIGURE 4.—Oil immersion photograph of cells containing chromophilic globules.

FIGURE 5.—70½ hr. *Rp* embryo showing the presence of degenerating cells in the distal portion of the nerve cord, which is double at this level, but has as many as eight canals more anteriorly.

FIGURE 6.—Transverse section through the cloacal region of a four day extreme Rumpless embryo. The nerve cord (n.c.) and notochord (n) are in the cloaca.

FIGURE 7.—75½ hr. *Rp* embryo. Sagittal section (high power) through the mid-ventral tail region showing the degenerate cells being extruded through the ectoderm.

distal tip of which is in contact with the other layers. The tail fold will form anterior to this point and anterior to the endodermal pouch.[1]

Degenerating cells: In embryos from both of our stocks cells in the region of fusion, described above, had many chromophilic granules. This is true for all three layers: the mesoderm had the most, the endoderm fewer, and the ectoderm the least. In virtually every embryo some of these cells (seemingly scattered from this focus) were present at the distal tip of the tail thickening. The concentration (amount and extent) of such cells in this region was variable (fig. 1–1a, b, c). Twelve of the 37 embryos from the *Rp* matings had more extensive concentrations than were observed in any of the normals; and in seven of these the granules were found in the adjacent nerve cord.

Second day. 30–49 hours

Earlier embryos were studied to determine any difference in the distribution of chromophilic granules. Embryos in this age group do not differ from the normal in other features. In normal embryos as young as 15 somites chromophilic granules were present at the posterior end of the remnant of the primitive streak. The earliest *Rp* embryos studied extensively were of the 16-somite stage; three of nine such embryos had chromophilic granules extending throughout the primitive streak and into the posterior portion of the nerve cord. In one case the concentration of granules was considerable. A few granules were found at the posterior region of one 12-somite embryo, while none were found in the one eight-somite embryo examined.

RECAPITULATION

Our observations lead us to the following synthesis of the genesis of dominant rumplessness: the tails are reduced or missing because of an early destruction of the material from which this structure is formed. The destruction, evidence of which is furnished by the presence of degenerating cells full of chromophilic granules, is begun some time toward the end of the second day of incubation and reaches its height by the end of the third day (when, in normal embryos, the most active growth in length of the tail is initiated). Sections of affected embryos at this stage reveal that the entire indifferent mass, the source of material for the pos-

[1] The nature of the endodermal pouch has been the occasion of some disagreement. LILLIE (1919) and others hold that it is the hind-gut. Recently GRUENWALD (1941) claims that it is a precociously formed allantoic diverticulum and that the hind-gut forms later when the tail-fold is formed and that the latter is a consequence of the folding. According to GRUENWALD, the allantoic diverticulum swings ventrally and forward and opens out; the region of fusion becomes the cloacal membrane. This gut pocket is present in embryos from both of our stocks. Our observations on a large number of closely timed embryos confirm GRUENWALD'S interpretation.

terior extension of the caudal structures, is in a condition of extreme degeneration and that the nerve cord may be in the same condition. Variations in the amount of tissue involved and the degree of degeneration have been encountered and may be correlated with variations in the final condition. Gross effects of this degeneration are found toward the end of the fourth day when all degrees of reduction of the tail may be observed. It is during this day that the greatest growth in length of the tail occurs normally. The most obvious internal effect of the absence of the indifferent mass is the failure of the somites to form. The notochord and the nerve cord are shortened and end abruptly. The former is thick and blunt and the latter is distorted at the distal end. The fact that the nerve cord is less irregular in the extremes than in the intermediates of the fourth day may be explained by an actual sloughing off of cells in the former; some of the nerve cords in extreme cases have a very eroded appearance. This is substantiated by the existence of greater irregularity of the nerve cord and the presence of numerous degenerate cells within the neural tissue of embryos diagnosed as extreme Rumpless at the end of the third day.

DISCUSSION

Various developmental roles have been ascribed to degenerating cells. According to ERNST (1926) they are associated with processes of fusion, separation, or dissolution of tissues. PETER (1936) doubts that they have any morphogenetic function. In regard to the scattered cells containing chromophilic granules PETER may be correct, but when one encounters masses of degenerating cells consistently in the same region and at the same time in development (for example, cloacal region of the chick embryo during the third day), one suspects that they may be concerned in local morphogenesis (BOYDEN 1918, 1922). Regardless of their part in normal development, it is quite evident that degenerating cells play a considerable role in the genesis of the Rumpless condition. The facts that they occur in great concentration at a critical time, that there is evidence of their being extruded from the body, and that the tissue in which they are concentrated (indifferent cell mass) is lacking in older stages all point to this conclusion.

The presence of degenerating cells is undoubtedly the result of some previous activity which results in cell death, but the nature of the underlying process is obscure as yet.

The degeneration of the presumptive tail tissue may be the result of an extension of a normal "degeneration process" to a region which is usually not involved. A portion of the indifferent presumptive tail mass in normal embryos does degenerate. BOYDEN (1922) states in this respect (p. 174), "It is well known that the tail in modern birds, and of fowl in

particular, is shorter than in Archaeornithes. It is conceivable that the degenerating primitive-streak mass in the tail of the chick embryo represents a persistence of material once utilized in tail-building but now superfluous." The condition found in our Rumpless embryos may result from an exaggeration of the destruction of "superfluous" tissue so that it includes more or less of the material involved in normal tail formation. Some mechanisms for restricting marked developmental degenerations (such as occur in insect and amphibian metamorphosis) undoubtedly exist, and it is not at all impossible that a mutation may change the protective processes so that they would be less effective. The modifiers found in our Rumpless stocks could be factors favoring the restricting mechanisms.

DU TOIT (1913) has emphasized the distorted nerve cords and thickened notochords in his rumpless embryos. We feel certain that they are secondary results of the destruction of the indifferent tail mass. Multiple canals in the caudal end of the nerve cord are common in embryos of birds and mammals. SCHUMACHER (1927) claims that this occurrence is sufficiently frequent that it cannot be regarded as a pathological condition but must be a normal manifestation of the manner in which the posterior portion of the nerve cord forms. HOLMDAHL (1933) has pointed out that the posterior part of the nerve cord arises from a solid mass of cells (indifferent tail mass or "Rumpfschwanzknospe") instead of forming from a plate of tissue. According to SCHUMACHER, it is typical for canals which form from solid masses to appear as several smaller canals, discrete at first, then merging after some time. In our material multiple canals were encountered frequently among both the Rumpless embryos and those from the normal Leghorn stock. Additional distortion and irregularity were found only in older (third and fourth days) embryos from the Rumpless stock and were associated with degenerating cells in the indifferent cell mass. It is probable that the later distortion of the nerve cord is not causally dependent on the initial condition of multiple canals but may be superimposed upon it. It probably results from an increase in cell number at this region (for mitotic activity is high) in the absence of sufficient space for orderly expansion. The thickening of the notochord is probably due to a similar sequence of events.

Rumplessness in chicks has been described as probably being due to a growth retardation at a critical period (DANFORTH 1932). This is definitely not the case for the dominant Rumpless animals. Their growth is normal throughout development, and the tail abnormality is already established at the time at which DANFORTH applied his temperature treatment.

The embryology of three allelic (or very closely linked) mutants affecting the tail of the house mouse has been thoroughly described (CHES-

LEY 1935; GLUECKSOHN-SCHOENHEIMER 1938a, 1938b, 1940). The first of these results is a short-tailed condition in the heterozygotes ($T+$). The homozygotes (TT) die in utero at about 11 days. A striking feature of these embryos is the presence of numerous chromophilic granules in the posterior region shortly before death. CHESLEY was uncertain whether these were secondary results of a moribund condition or whether they were the cause of the degeneration. The other two mutants (t^1 and t^0) are expressed when combined with the T gene and yield only one type of surviving offspring, the tailless mice (Tt^1 and Tt^0). The t^0t^0 embryos die shortly after implantation, and the t^1t^1 embryos die before implantation.

It is interesting that genes having such similar results (tail reduction) in two different animals produce their effects by such dissimilar means. For, the tail reduction in surviving mice is effected by the degeneration of either part of or the entire tail after it had formed normally until about the 11th day of embryonic development. At this time a constriction occurs at a given region of the tail, and all of the tissues distal to this degenerate and form a filament which eventually is sloughed off. The most obviously abnormal structure in all of the heterozygotes is the notochord; this structure is interpreted by the quoted authors as probably playing a causal role in the etiology of tail reduction.

Tail reduction due to the dominant gene *Rp* differs from the above in that: (1) There is no lethal action in the homozygotes. All of the gene effect appears to be localized. (2) The presumptive and not the definitive tail tissue degenerates, and the distal somites do not form. (3) The notochord does not have a causal role; its deformity (as well as that of the nerve cord) is a secondary result of the absence of its formative material.

SUMMARY

A study of dominant Rumpless embryos reveals that tail reduction is completed by the end of the fourth day of development. This confirms DU TOIT'S findings.

At this time there is a variation in the amount of tail reduction that corresponds with a similar variation (probably the result of genetic modifiers) found in adults.

Sections of earlier embryos show that the tail reduction results from a degeneration of the presumptive tail tissue. Degeneration is initiated toward the close of the second day and is most pronounced during the third day of development.

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CORRIGENDA

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Page 286, line 24, insert "Kyoto, Japan" between "New Jersey" and "Caddo Lake."

Page 304, table 1, for genotypic constitution of Smyrna I read "Xsxs" instead of "XsXs."

Page 314, the unnumbered table should follow immediately after paragraph 2, line 22 under "Discussion of Results," page 313.

Page 345, line 18, for "both dominants are" read "one or both dominants is."

Page 345, line 22, for "both" read "one or both."

Page 464, figure 1, in the diagram of the fourth chromosome cross the white chromosome in the F_1 generation should contain the genes *inc* and *hk*.

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